National Library of Canada

Acquisitions and Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada

Direction des acquisitions et des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file - Votre référence

Our file - Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

Intestinal Chiral Inversion of 2-Arylpropionic Acids

by

Saeed Sattari

A thesis submitted to the **Faculty of Graduate Studies and Research** in partial fulfillment of the requirement for the degree of Master of Science

IN

Pharmaceutical Sciences

(Pharmacokinetics)

Faculty of Pharmacy and Pharmaceutical Sciences

EDMONTON, ALBERTA

Fall 1993

National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file *Votre référence*

Our file *Notre référence*

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-88263-8

Canada

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: **Saeed Sattari**

TITLE OF THESIS: **Intestinal Chiral Inversion of 2-Arylpropionic Acids**

DEGREE: **M. Sc.**

YEAR THIS DEGREE GRANTED: **Fall 1993**

Permission is hereby granted to the **University of Alberta Library** to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

S. Sattari

PERMANENT ADDRESS:

#7 Takestan Ave

Satarkhan St, Tehran 14

Iran

Date: June 15, 93

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESAERCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **"Intestinal Chiral Inversion of 2-Arylpropionic Acids"** submitted by **Saeed Sattari** in partial fulfillment of the degree of Master of Science in Pharmaceutical Sciences (**Pharmacokinetics**).

Dr. F. Jamali (Supervisor)

Dr. F. M. Pasutto (Professor)

Dr. Y. K. Tam (Professor)

Dr. A. S. Russell (Professor)

Date: June 15, 93

To my wife **Fariba** and my children

Somayeh and **Alireza**

for their

understanding, support and patience

ABSTRACT

In the presence of excised human and rat gut, the pharmacologically less active R isomers of both ibuprofen (IB) and fenoprofen (FN) are bioinverted to their pharmacologically active S antipodes. In addition, in humans, the extent of presystemic inversion of IB was found to be positively correlated with the Tmax of the oral dosage form. In an attempt to localize the intestinal site of inversion, we incubated R-FN in a glass organ bath (at 37°, in continually oxygenated Krebs-Henseleit (K-H) buffer pH 7.4 for 3 h) in the presence of intestinal contents, epithelium or muscle layer, and everted jejunum sacks of antibiotic treated (ABT) (500 mg/kg of erythromycin and neomycin b. i. d. for 3 days) and control rats (adult Sprague-Dawley). R-IB was also incubated in organ bath in the presence of everted rat jejunum, ileum and colon and freshly excised (intact and/or homogenized) human ileum and colon. Absorption rate dependency of IB inversion was examined in rats by giving 20 mg/kg of racemate as an aqueous solution, suspension, or sustained release granules. Utilizing stereospecific assays for IB and FN, samples were analyzed for intact and glucuronidated enantiomers.

The extent of inversion of R-FN by the everted jejunum was substantial. No significant differences between the control (36.5±4.4%) and ABT rats (37.0±2.1%) were observed. The intestinal contents showed little inversion activity (5.7±2.2%). The epithelial cells were found to be the major site of inversion in the small intestine wall (37.5±4.7%) with the minor contribution from muscle layer to the inversion process (7.8±2.1%). The extent of R-IB glucuronidation was found to be 15.5±1.5%, 21.2±4.7%, 14.5±2.8%, 27.0±6.2% and 18.1±8.6% for intestinal contents, epithelial

cells, muscle layer, and control and ABT rats, respectively. R-IB was inverted by rat jejunum (12.2±1.6%), ileum (14.2±2.0%) and colon (4.4±0.6%). In addition, jejunum and ileum stereoselectively glucuronidated IB (R, 9.7±3.2; S, 19.5±2.4%) and (R, 8.2±3.6; S, 22.3±3.7%), respectively. Glucuronidation was not stereoselective in colon (R, 20.2±3.0; S, 21.2±3.9%). Excised human ileum (33%) and two colons (20% and 34.5%) were able to invert R-IB. The conjugation was not evident for ileum but was noticeable for colons (R, 20.0% and 23.3% and S, 11.5% and 13.0%, respectively). Inversion activity was not found using freshly homogenized human ileum and colon. In vivo, the S:R AUC ratios after administration of a sustained release IB formulation (7.3±1.5) were significantly higher than suspension (3.6±1.1) and solution (3.5±0.22). A positive and linear correlation was found when S:R AUCs were plotted against Tmaxs of R-IB (r=0.82).

In conclusion, the epithelium layer of the rat gut is the main site of intestinal chiral bioinversion of FN. However, the intestinal contents and the muscle layer are able to conjugate FN. The extent of inversion following administration of oral dosage formulation of IB is significantly and positively correlated with the Tmax of formulation. Different segments of rat intestine and also human colon and ileum are capable of performing bioinversion and glucuronidation of IB.

ACKNOWLEDGEMENTS

The author wishes to thank **Dr. F. Jamali** for his patience, guidance, encouragement and superb supervision of the project both during the experimental lab work and writing-up stages. The author would also like to thank members of the supervisory committee **Dr. F. M. Pasutto, Dr. Y. K. Tam and Dr. A. S. Russell** for their suggestions and guidance.

The financial support of the **Iranian Ministry of Health** during the course of these investigations is also gratefully acknowledged.

The author wishes to thank Mr. **Derrick Freitag** for his val
able help in preparing microbial culture media.

TABLE OF CONTENTS

Chapter	Page
1.Introduction	1
1.1. 2-Arylpropionic Acids	2
1.2. Chiral Bioinversion	2
1.3. Site of Inversion	5
1.4. Mechanism of Inversion	8
1.5. Extent of Inversion	9
1.6. Physicochemical Inversion	11
1.7. Rationale	13
1.8. Hypothesis	14
1.9. Objectives	14
1.10. References	15
2. Chiral Inversion and Conjugation of R-(-)-Fenoprofen by the Rat Gut	21
2.1. Introduction	22
2.2. Materials and Methods	23
2.2.1. Chemicals	23
2.2.2. Animals and Treatments	23
2.2.2.1. Rat Surgery and Tissue Preparations	23
2.2.2.2. Isolation of Epithelial Cells	23

2.2.2.3. Antibiotic Treated (ABT) and Control Rats 25

2.2.2.4. Culture Media Preparations 25

2.2.2.5. In vivo Incubation and Sampling 26

2.2.3. Assay and Sample Analysis 26

2.2.4. Data Analysis 26

2.3. Results 27

2.4. Discussion 27

2.5. Conclusion 33

2.6. References 34

3. Absorption Rate Dependency of Ibuprofen Inversion

in the Rat 38

3.1. Introduction 39

3.2. Materials and Methods 40

3.2.1. Chemicals 40

3.2.2. In vivo Study 40

3.2.2.1. Preparation of Dosage Forms 40

3.2.2.2. Animals, Dosing and Sampling 41

3.2.3. In vitro Study 41

3.2.3.1. Animals and Tissue Preparations 41

3.2.3.2. In vitro Incubation of R-IB with Rat Intestine 42

3.2.3.3. In vitro Incubation of R-IB with Human Gut 42

3.2.4. Stereospecific Assay for IB Enantiomers 43

3.2.4.1. Standard Solutions 43

3.2.4.2. Sample Preparations 44

3.2.4.3. Apparatus and Chromatographic Conditions 44

3.2.5. Data Analysis 45

3.3. Results 46

3.3.1. In vivo Study 46

3.3.2. In vitro Study 46

3.4. Discussion 52

3.5. Conclusion 57

3.6. References 58

4. General Discussion and Conclusions 62

List of Tables

		Page
Table 1-1.	The relative in vitro and in vivo potencies of some chiral 2-APAs.	6
Table 1-2.	The extent of inversion of 2-APAs in different species.	12
Table 3-1.	The pharmacokinetic indices of IB after oral administration of 20 mg/kg of racemate as solution, suspension, or sustained release formulations.	48

List of Figures and Illustrations

		Page
Scheme 1-1.	The family of NSAIDs.	3
Figure 1-1.	Chemical structures of some chiral 2-APAs.	4
Figure 1-2.	The correlation between Tmax and the S:R AUC ratios for IB oral dosage form.	7
Figure 1-3.	A proposed mechanism of chiral bioinversion.	10
Figure 2-1.	Percent in vitro inversion and glucuronidation of R-FN in the rat gut.	28
Figure 2-2.	Percent of initial concentrations of R-FN found as intact and conjugated form in the jejunum of control and ABT rats.	29
Figure 2-3.	Ratios of conjugated:intact FN found in the rat gut.	32
Figure 3-1.	The time course of IB enantiomers after oral administration of 20 mg/kg of racemate in three different dosage forms.	47
Figure 3-2.	The correlation between the Tmax of R-IB and the extent of chiral inversion in the rat.	49
Figure 3-3.	The extent of in vitro inversion of R-IB by the rat intestine.	50
Figure 3-4.	The extent of in vitro conjugation of R- and S-IB in the rat intestine.	51
Figure 3-5.	Inversion and conjugation of R-IB in the presence of an excised segment of human colon.	53

Abbreviations

ABT	Antibiotic treated rats
ACN	Acetonitrile
α	Statistical significance level
ANOVA	Analysis of variance
2-APAs	2-Arylpropionic Acids
AUC	Area under plasma concentration-time curve
b. i. d.	Twice daily
ß	Elimination rate constant
Cmax	Maximum plasma concentration.
CoA	Co-enzyme A
°	Degrees Celsius
ECF	Ethyl chloroformate
FN	Fenoprofen
h	Hour(s)
HPLC	High-performance liquid chromatography
HTA	Hydratropic acid
Hz	Hertz
IB	Ibuprofen
IS	Internal standard
i.v.	Intravenous
kg	Kilogram
K-H	Krebs-Henseleit

KT	Ketoprofen
L	Liter(s)
mg	Milligram
mg/L	Milligram per liter
min	Minutes(s)
mL	Milliliter(s)
n	Number of observations
NA	Nutrient agar
nm	Nanometre
NSAIDs	Non-steroidal anti-inflammatory drugs
PCA	Plate count agar
PEA	(R)-(+)-1-(1-phenyl)ethylamine
p.o.	Oral
r	Correlation coefficient
SD	Standard deviation
SR	Sustained release
SRS-A	Slow release substance of anaphylaxis
TEA	Triethylamine
$t_{1/2}$	Half life
Tmax	Time to peak plasma concentration
μL	Microliter(s)
UV	Ultraviolet

CHAPTER ONE

INTRODUCTION

1. Introduction

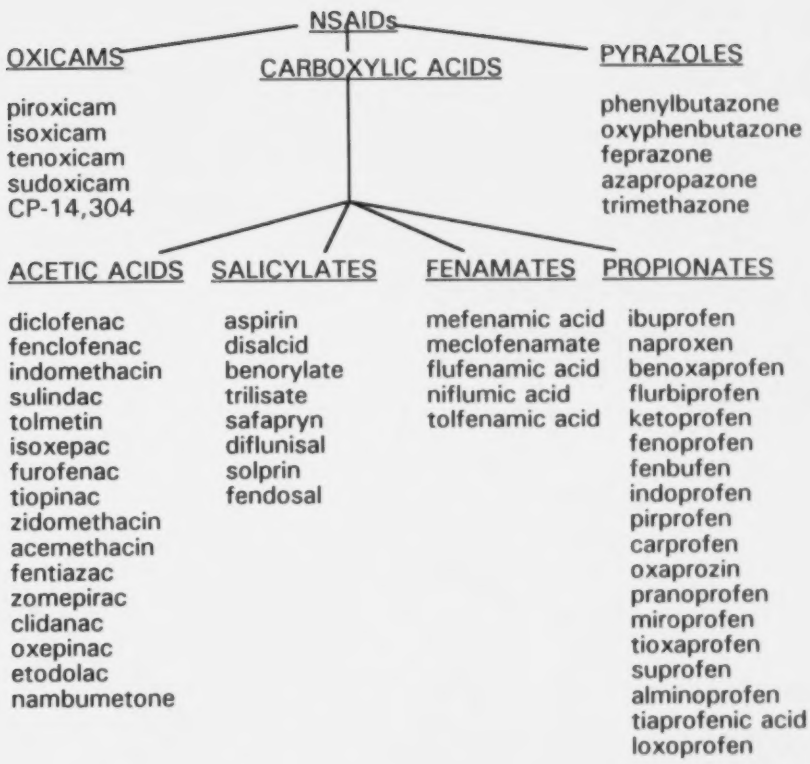
1.1. 2-Arylpropionic acids

The non-steroidal anti-inflammatory drugs (NSAID) show significant clinical effects in the treatment of chronic arthropathies such as rheumatoid and osteoarthritis. NSAIDs are being used to alleviate the symptoms and the pain associated with chronic inflammatory diseases. NSAIDs are a structurally diverse pharmacological class of compounds including carboxylic acid derivatives (salicylates, acetic acids, fenamates, propionic acids), pyrazoles and oxicams (scheme 1-1).

The 2-arylpropionic acid derivatives (2-APAs), which are a very important sub-group of NSAIDs, possess a single chiral carbon in the propionic acid side chain. As a result, they have two enantiomers. Figure 1-1 depicts the chemical structure of some chiral 2-APAs. Although the pharmacological properties of 2-APAs (prostaglandins synthetase inhibition and anti-inflammatory effects) reside almost entirely with the S-(+)-enantiomer (1) they are currently administered as racemates with the exception of naproxen.

1.2. Chiral bioinversion of 2-APAs

In 1922, Key and Raper (2) isolated predominantly S-(+)- configuration of hydratropic acid (HTA) from dog urine after parenteral administration of the racemate. Thirty-three years later in 1955, Robinson et al. (3) found excess S-(+)-HTA in urine of rabbits after giving the racemate. These authors proposed that "partial resolution" of racemic HTA occurred in the mammals. In fact, it was the discovery of a new pathway



Scheme 1-1: The family of NSAIDs, a structurally diverse class of compounds.

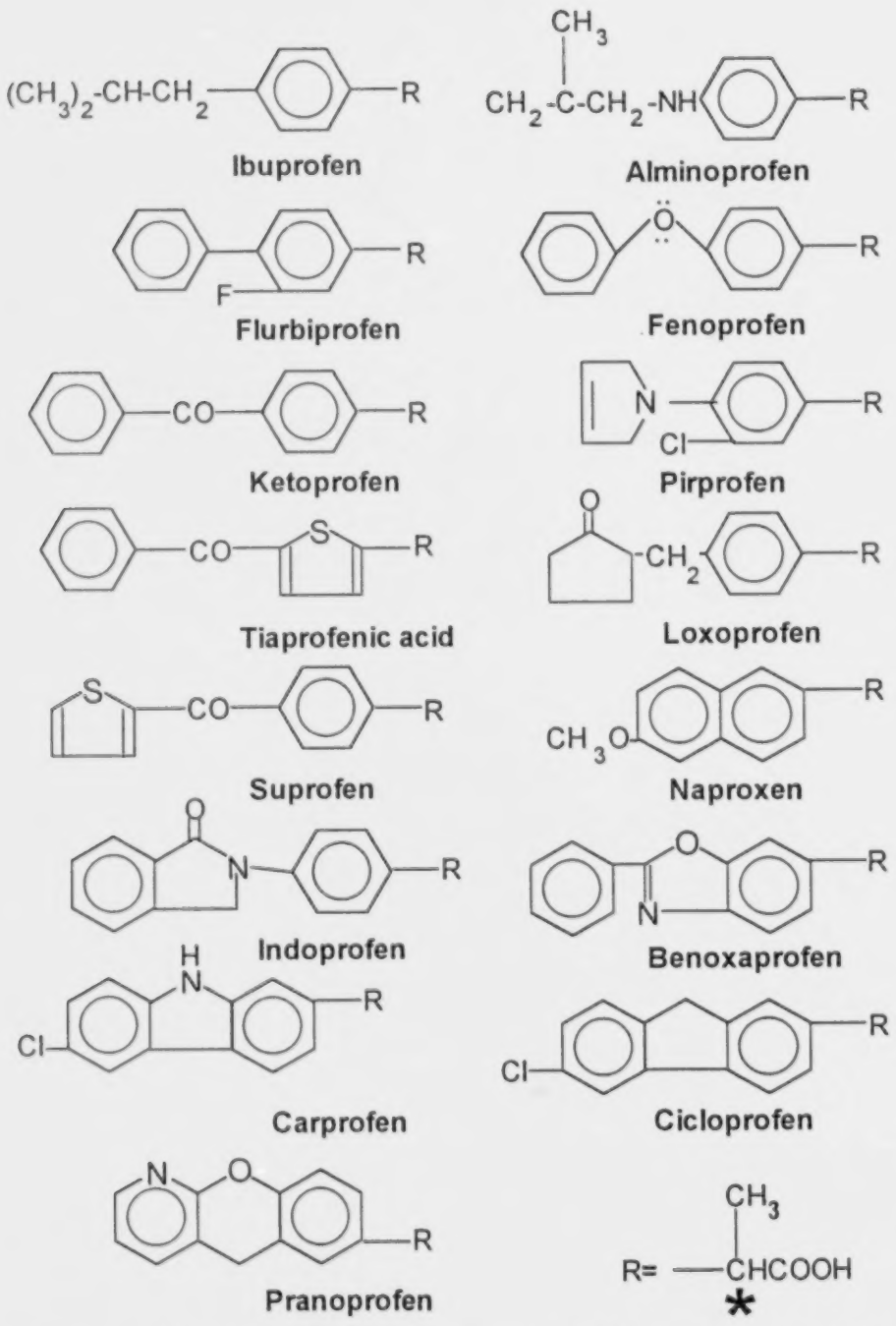


Figure 1-1; Chemical structure of some chiral 2-arylpropionic acids of NSAIDs. *, Indicates the chiral centre.

of metabolism for HTA. The unique feature of the biotransformation of the 2-APAs is the metabolic chiral inversion at the asymmetric centre. After adminstration to humans and laboratory animals, many members of 2-APAs, such as ibuprofen (IB), fenoprofen (FN) and benaxoprofen, undergo a unique and unidirectional chiral inversion in which the R enantiomers are bioinverted to the S enantiomer (1, 4). This explains why stereochemically pure R isomers exhibit pharmacological activities in vivo but not in vitro (5) as shown in Table 1-1.

As other investigators have shown, inversion activity is more likely to be detected when intact tissues or cells are used instead of sub-cellular fractions (6 ,7, 8, 9). It has been shown that in vitro inversion is dependent upon a variety of factors such as the drug and its concentration, species used, method of tissue preparation, cell density, presence of oxygen and co-factors, composition of incubation medium and the incubation time (10, 11).

1.3. Site of Bioinversion

The site of inversion has been a matter of debate during the past several years. The formation of S-IB after i. v. injection of optically pure R-IB is indicative of the existence of a systemic inversion (12). On the other hand, higher S:R AUC ratios obtained after oral administration of slowly absorbed IB tablets to humans (34, 36) (Fig. 1-2) and oral administration of ketoprofen (KT) to rats, as compared to the i.v. route (14), indicate that the gastrointestinal tract (GIT) is partly responsible for presystemic

Table 1-1: The relative in vitro and in vivo potencies of some chiral 2-APA NSAIDs (Ref. 6).

Compound	S/R activity in vitro	S/R activity in vivo	Test system
Carprofen	>16		PG synthetase inhibition (sheep)
	>23		Platelet aggregation
		>16	Gastric ulcer score
		13.5	Acute adjuvant arthritis (rat)
Fenoprofen	35	1	PG synthetase inhibition (human platelet)
Flurbiprofen	878		Antagonism of rat SRS-A on the tracheal chain of guinea-pigs
		2-16	Guinea-pig anaphylaxis
Ibuprofen	160		PG synthetase inhibition (bovine)
		1.4	Acetylcholine writhing (mouse)
		1.4	Pain threshold (rat)
		1.1	UV erythema (guinea pig)
Indoprofen	100		PG synthetase inhibition
		18.3	Carrageenin-induced oedema (rat)
		31.4	Granuloma pouch (rat)
		23.1	Phenylquinone writhing (mouse)
Naproxen	133		PG synthetase inhibition (sheep)
	70		PG synthetase inhibition (bovine)
		27.5	Anti-inflammatory activity (rat paw oedema)
		14.7	Antipyretic activity (rat)
Pirprofen	6.4	-	PG synthetase inhibition (sheep)

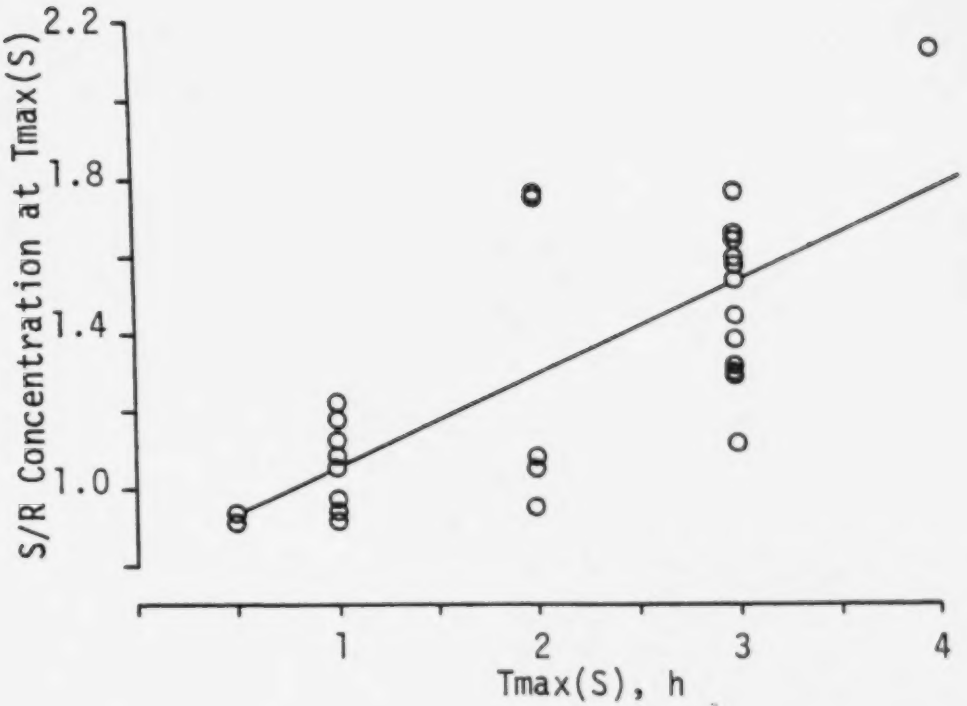


Figure 1-2; The relationship between the peak plasma concentration ratios of ibuprofen enantiomers and the Tmax of S-IB (r=0.74) following single 600 mg oral racemic doses of various formulations with different absorption rates (Ref. 15).

inversion of these xenobiotics.

The GIT, liver, and kidney have been identified as sites of chiral inversion for 2-APAs (9, 15, 16). Hence, bioinversion may take place in the GIT and liver during the absorption phase, before the drug reaches the systemic circulation and/or in the kidney and liver after the absorption phase is completed. The ability of other tissues or organs to effect chiral inversion has not been thoroughly studied.

1.4. Mechanism of inversion

The R to S inversion that occurs for some members of 2-APAs may have both therapeutic and toxicologic implications (17). Although there are many articles in literature concerning chiral inversion of 2-APAs, the mechanism (s) has not been completely delineated (8, 18, 19).

In 1974 Wechter et al. (19) proposed a mechanism for inversion of R-IB in humans. In recent years the mechanism of this unique metabolic pathway has been studied by many investigators (8, 16, 18, 20). Briefly, the mechanism of inversion process appears to be as follows: R-IB is stereospecifically converted to its Co-enzyme A (CoA) thioester by long chain acyl CoA synthetase in the presence of ATP, CoA, and Mg^{+2} and this is the rate-limiting step. Consequently, the R-CoA thioester undergoes racemization of the propionic acid moiety by a non-stereoselective racemase to form the S-CoA thioester. The formed R- and S-CoA thioesters are stereoselectively cleaved by a hydrolase to release R-(-) and S-(+)- enantiomers. The aforementioned process, which

was proposed by Nakamura et al. (9) has become the accepted mechanism in bioinversion of 2-APAs. By studying subcellular fractions from rat liver, it has been shown that thioester formation occurred in mitochondria and microsomes but not in cytosol (21). Figure 1-3 illustrates the schematic explanation for chiral bioinversion.

An alternative metabolic pathway for R and S-CoA thioester is acyl transfer to produce hybrid triglycerides resulting in incorporation of 2-APAs into the lipid and adipose tissues (22). This process would result in accumulation in lipids and possibly lead to central nervous system toxicity due to disruption of lipid metabolism and membrane function (24, 25, 26).

Another proposed mechanism for chiral bioinversion is the formation of oxidative products or non-enzymatic dissociation of the α methine (8) which have been since discounted (9).

1.5. Extent of inversion

The extent of in vivo inversion of 2-APAs is dependent on the drug, dose, route of administration, absorption rate of formulation, and the species used (15). Jamali et al. showed that while the extent of in vivo inversion was independent of the size of the dose, it was positively correlated with the Tmax of the dosage form (33).

The extent of in vitro inversion of these xenobiotics is influenced by the drug and its concentration, species and tissues used, method of tissue preparation, cell density, co-factors, incubation media and duration of incubation, presence of oxygen and

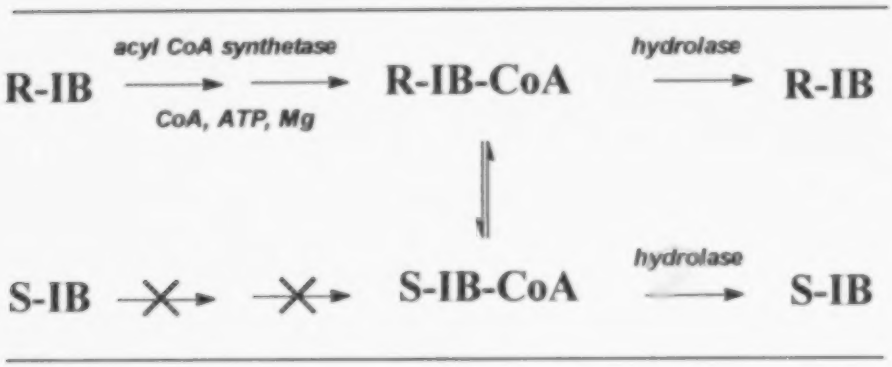


Figure 1-3; Proposed mechanism of inversion.

temperature (9, 11). Concomitant use of other medications such as phenobarbital, probenecid, clofibrate (27, 28) and pathological conditions, such as renal failure (29), also may have some impact on the extent of bioinversion.

There is a significant interspecies variability in the extent of in vivo R to S inversion for 2-APAs. It can be vary from 0 to 10% (flurbiprofen and tiaprofenic acid in humans and rats), 50-70% (IB in humans, rats and dogs) and almost complete inversion in the case of FN in the rat. In humans, KT only undergoes 10% inversion while in rats 80% R to S inversion occurs (14, 15). These results indicate the specificity of enzymes in different species. Table 1-2 illustrates the extent of inversion of some chiral 2-APAs in different animals.

1.6. Physicochemical inversion

Chiral inversion may also occur for some chiral drugs (moxalactam, oxazepam) in the absence of enzymes (30). Factors, such as storage and the presence of other compounds may accelerate the chiral inversion. Contrary to metabolic inversion, chemical conversion is not usually unidirectional (37). Chemical chiral inversion has been reported for ibutilide, an investigational class III anti-arrhythmic drug through a mixed SN_2 and SN_1 reaction at 80^o in aqueous solution (31). These results indicate the importance of enantiomer stability in liquid dosage forms when a single enantiomer is

Table 1-2: The extent of inversion of 2-APAs in different species.

2-APA	%R to S inversion	species	Reference
benoxaprofen	100%	rat	40
clidanac	extensive	guinea pigs	15
fenoprofen	90%	rat, human, rabbit	27, 4
flurbiprofen	0-10%	human	15
	20%	rat	15
ibuprofen	50-75%	human	6, 35, 15
	50-65%	rat	41, 42
	70-75%	dogs	32
ketoprofen	10%	human,rabbit	15
	56-80%	rat	14, 43, 15
tiaprofenic acid	0%	human, rat	15

used as the pharmacologically active agent.

Ahn et al. have proposed that the degree of racemization was affected by ethyl chloroformate (ECF) and derivatization agents for IB (38). This issue has been examined in detail by Wright and Jamali (39). These authors have shown that ECF concentration influences the extent of stereochemical conversion of S-IB, S-KT, S-flurbiprofen and R-etodolac. However, they have observed that this would not produce a significant analytical error in the assay procedures as high concentrations of ECF minimized the stereochemical conversion.

1.7. Rationale

Substantial presystemic bioinversion has been reported for IB and FN in humans (33, 15), and benoxaprofen (7) and FN in rats (4). The GIT has been shown to be the site of presystemic inversion for 2-APAs (34, 35). The inversion activity of the GIT can be due to the involvement of the intestinal contents, the epithelial cells and/or muscle layer of the intestinal wall. It is not clear which is mainly responsible for the chiral bioinversion and conjugation of chiral 2-APAs. Rats were used as an animal model and FN as the test drug because FN showed similar pharmacokinetics in both humans and rats (15) and also because it undergoes substantial inversion in the rat gut(4).

Previous studies (33, 34, 36) revealed that the extent of inversion for orally administered IB was dependent upon the plasma Tmax of the dosage form. This issue has not been studied in the rat. The stereoselectivity in pharmacokinetic parameters of IB,

as well as the R to S inversion, have been demonstrated to have the same pattern in humans and rats (15). Thus, the rat may serve as a suitable animal model in order to study the pharmacokinetics of IB enantiomers after oral administration of dosage forms with different dissolution rates. In addition, there is no direct evidence in literature proving that human gut is capable of inverting IB.

1.8. Hypotheses

1. The intestinal epithelium layer in the rat gut is the site of chiral bioinversion.
2. The extent of bioinversion is positively correlated with the Tmax of oral dosage forms of IB.
3. The human and rat intestine are capable of inverting R-IB to its S antipode.

1.9. Objectives

1. To investigate and localize the site of chiral inversion in the rat intestine.
2. To utilize three IB oral dosage forms, with different rates of absorption in order to establish the correlation between the rate of absorption and the extent of chiral inversion.
3. To provide direct evidence for the capability of human and rat intestine to perform chiral bioinversion.

1.10. References

1. A. J. Hutt and J. Caldwell: The metabolic chiral inversion of 2-arylpropionic acids; a novel route with pharmacological consequences. J. Pharm. Pharmacol. 35, 693-704 (1983).

2. H. D. Key and H. S. Raper: Predominating dextrorotatary hydratropic acid was isolated from dog urine after parenteral administration of the racemic mixture. Biochem. J. 16, 465 (1922).

3. D. Robinson, J. N. Smith and R. T. Williams: Predominating dextrorotatary hydratropic acid was found from rabbit urine after oral administration of the racemic mixture. Biochem. J. 59, 153 (1955).

4. B. W. Berry and F. Jamali: Presystemic and systemic inversion of R-(-)-fenoprofen in the rat. J. Pharmacol. Exp. Ther. 258, 695-701 (1991).

5. S. S. Adams, P. Bresloff, C. G. Mason: Pharmacological differences between the optical isomers of ibuprofen: evidence for metabolic inversion of the (-)-isomer. J. Pharm. Pharmacol. 28, 256-257 (1976).

6. A. J. Hutt and J. Caldwell: The importance of stereochemistry in clinical pharmacokinetics of the 2-arylpropionic acid non-steroidal anti-inflammatory drugs. Clin. Pharmacokin. 9, 371-373 (1984).

7. R. G. Simmonds, T. J. Woodage, S. M. Duff and J. N. Green: Stereospecific inversion of R-(-)-benoxaprofen in rat and man. Eur. J. Drug Metab.

Pharmacokin. 5, 169-172 (1980).

8. J. M. Mayer, C. Bartolucci, J. M. Maitre and B. Testa: Metabolic chiral inversion of anti-inflammatory 2-arylpropionates: lack of inversion in liver homogenates, and study of methine proton acidity. Xenobiotica. 18, 533-543 (1988).

9. Y. Nakamura and T. Yamaguchi: Stereoselective metabolism of 2-phenylpropionic acids in rat. I. In vitro studies on stereoselective isomerization and glucuronidation of 2-phenylpropionic acids. Drug Metab. Dispos. 15, 529-539 (1987).

10. M. Mouelhi, H. W. Relius, C. Fenselau and D. M. Dulik: Species-dependent enantioselective glucuronidation of three 2-arylpropionic acids. Naproxen, Ibuprofen and Benoxaprofen. Drug Metab. Dispos. 15, 767-772 (1987).

11. S. M. Sanius, W. J. Adams, D. G. Kaiser, G. W. Halsted and T. A. Baillie: Studies on the metabolism and chiral inversion of Ibuprofen in isolated rat hepatocytes. Drug Metab. Dispos. 18, 527-533 (1990).

12. S. R. Cox: Effect of route of administration on chiral inversion of R-(-)-Ibuprofen. Clin. Pharmacol. Ther. 43, 146 (1988).

13. E. J. D. Lee, K. Williams, R. Day and G. Graham: Stereoselective disposition of ibuprofen enantiomers in man. Br. J. Clin. Pharmacol. 19, 669-674 (1985).

14. R. T. Foster and F. Jamali: Stereoselective pharmacokinetics of Ketoprofen in the rat: Influence of route of administration. Drug Metab. Dispos. 16, 623-626 (1988).

15. F. Jamali: Pharmacokinetics of enantiomers of chiral non-steroidal anti-inflammatory drugs. Eur. J. Drug Metab. Pharmacokin. 13, 1-9 (1988).

16. C. S. Chen, W. R. Shieh, P. H. Lu, S. Harriman and C. Y. Chen: Metabolic chiral inversion of Ibuprofen in mammals. Biochem. Biophys. Acta. 1078, 411-417 (1991).

17. K. Williams, R. Day, R. D. Knihinicki and A. Duffield: The stereoselective uptake of ibuprofen into adipose tissue. Biochem. Pharmacol. 35, 3403-3405 (1986).

18. R. D. Knihinicki, K.M. Williams and R. O. Day: Chiral inversion of 2-arylpropionic acid non-steroidal anti-inflammatory drugs: In vitro studies of Ibuprofen, Flurbiprofen. Biochem. Pharmacol. 38, 4389-4395 (1989).

19. W. J. Wechter, D. G. Loughhead, R. J. Reischer, G. J. VanGiessen and D. G. Kaiser: Enzymatic inversion at saturated carbon: Nature and mechanism of the inversion of R-(-)-p-isobutyl hydratropic acid. Biochem. Biophys. Res. Commun. 61, 833-837 (1974).

20. S. M. Soglowek. G. Geisslinger, J. Mollenhauer and K. Brune: Metabolic chiral inversion of 2-arylpropionates in rat H4IIE and human HEPG2 hepatoma cells. Biochem. Pharmacol. 43, 1487-1492 (1992).

21. M. P. Knadler and S. D. Hall: Stereoselective arylpropionyl-CoA thioester formation in vitro. Chirality 2, 67-73 (1990).

22. K. Williams, R. Day, R. D. Knihinicki?and A. Duffield: The stereoselective uptake of Ibuprofen enantiomers into adipose tissue. Biochem. Pharmacol. 35, 3403-3405 (1986).

23. A. Avgerinos and A. J. Hutt: Interindividual variability in the enantiomeric disposition of Ibuprofen following the oral administration of the racemic drug to healthy volunteers. Chirality 2, 249-256 (1990).

24. B. C. Sallustio, P. J. Meffin and K. M. Knights: The stereoselective incorporation of fenoprofen into rat hepatocyte and adipocyte triacylglycerols. Biochem. Pharmacol. 37, 1919-1923 (1988).

25. R. S. Tracy and S. D. Hall: Metabolic inversion of (R)-ibuprofen: epimerization and hydrolysis of ibuprofenyl-Coenzyme A. Drug Metab. Dispos. 20, 322-327 (1992).

26. R. Fears, K. H. Baggaley, R. Alexander, B. Morgan and R. M. Hindley: The participation of ethyl 4-benzyloxybenzoate (BRL 10894) and other aryl-substituted acids in glycerolipid metabolism. J. Lipid Res. 19, 3-11 (1978).

27. P. J. Hayball, and P. J. Meffin: Enantioselective disposition of 2-arylpropionic acid anti-inflammatory drugs III. Fenoprofen. J. Pharmacol. Exp. Ther. 240, 631-636 (1987).

28. K. M. Knights, T. F. Addinall, B. J. Roberts: Enhanced chiral inversion of R-Ibuprofen in liver from rats treated with clofibrate. Biochem. Pharmacol. 41, 1775-1777 (1991)

29. A. Abas and P. J. Meffin: Enantioselective Disposition of 2-arylpropionic acid non-steroidal anti-inflammatory drugs. IV. Ketoprofen disposition. J. Pharmacol. Exp. Ther. 240, 637-641 (1987).

30. F. Jamali, R. Mehvar and F. M. Pasutto: Enantioselective aspects of drug action

and disposition: Therapeutic pitfalls. J. Pharm. Sci. 78, 695-715 (1989).

31. W. J. Lambert and P. G. Timmer: Racemization of Ibutilide in solution: A factor to consider when choosing to develop the racemate or a single enantiomer. Pharm. Res. 8, 1444-1447 (1991).

32. H. Y. Ahn, G. L. Amidon and D. E. Smith: Stereoselective systemic disposition of Ibuprofen in the dog. Pharm. Res. 8, 1186-1190 (1991).

33. F. Jamali, R. Mehvar, A. S. Russell, S. Sattari, W. W. Yakimets and J. Koo: Human pharmacokinetics of Ibuprofen enantiomers following different doses and formulations: Intestinal chiral inversion. J. Pharm. Sci. 81, 221-225 (1992).

34. F. Jamali, N. N. Singh, F. M. Pasutto, A. S. Rusell and R. T. Coutts: Pharmacokinetics of Ibuprofen in humans following oral administration of tablets with different absorption rates. Pharm. Res. 5, 40-43 (1988).

35. R. Mehvar and F. Jamali: Pharmacokinetic analysis of the enantiomeric inversion of chiral non-steroidal anti-inflammatory drugs. Pharm. Res. 5, 76-79 (1988).

36. M. T. Mortin, S. R. Cox, J. M. Hageman, and G. J. Szpunar: Serum concentrations of Ibuprofen enantiomers following multiple dose administration of sustained release Ibuprofen 800 mg tablets or Motrin 400 mg tablets. J. Pharm. Res. 10, (Suppl.) S59 (1988).

37. J. Gal: Indirect chromatographic methods for resolution of drug enantiomers-Synthesis and separation of diastereomeric derivatives, In Drug Stereochemistry: Analytical methods and pharmacology. Eds, W. I. Wainer and D. E. Drayer.

New York: Marcel Dekker, pp, 81-113 (1988).

38. H. Y. Ahn, S. T. Wu, G. K. Shiu, T. D. Doyle, C. A. Brunner and W. F. Trafton: Potential racemization during enantiomeric analysis of ibuprofen. Pharm. Res. 8, (Suppl.) S31, (1991).

39. M. R. Wright and F. Jamali: Limited extent of stereochemical conversion of chiral NSAIDs induced by derivatization method employing ethyl chloroformate (in press J. Chromatogr. 1993)

40. R. G. Simmonds, T. J. Woodage, S. M. Duff and J. N. Green: Stereospecific inversion of (R)-(-)-benoxaprofen in rat and man. Eur. J. Drug Metab. Pharmacokin. 5, 169-172 (1980).

41. R. D. Knihinicki, R. O. Day, G. G. Graham and K. M. Williams: Stereoselective disposition of ibuprofen and flurbiprofen in rats. Chirality 2, 134-140 (1990).

42. C. S. Chen, T. Chen and W. R. Shieh: Metabolic stereoisomeric inversion of 2-arylpropionic acids. On the mechanism of ibuprofen epimerization in rats. Biochem. Biophys. Acta. 1033, 1-6 (1990).

43. S. Iwakawa, S. Hashimoto, L. Z. Benet and E. T. Lin: Stereoselective disposition of ketoprofen in rats. Drug Metab. Dispos. 19, 717-718 (1991).

CHAPTER TWO

Chiral Inversion and Glucuronidation of R-(-)-Fenoprofen in the Rat Gut

2.1. Introduction

The non-steroidal anti-inflammatory drugs (NSAID) show significant clinical effects in the treatment of rheumatoid and osteoarthritis. 2-Arylpropionic acids (2-APA) are a main sub-group of NSAIDs. These drugs (e.g. fenoprofen, FN) have an asymmetric carbon in the propionic acid side chain in α position to the carboxyl group. Therefore, they are often available and used as racemates. However, the pharmacological activities of 2-APAs are attributed mainly to the S enantiomers (1,2). A unique metabolic pathway for some chiral 2-APA is a unidirectional bioinversion whereby the less active R enantiomer is inverted to the active S antipode (3,4). This explains why the R isomers of some 2-APAs exhibit pharmacological activity in vivo but not in vitro.

In vivo, the chiral inversion takes place at various sites. The formation of S-ibuprofen after i. v. administration to human and rat of R-ibuprofen (5, 6) is strong evidence for systemic inversion, perhaps in the liver, as has been shown for FN in the rat (7). On the other hand, higher S:R AUC ratios observed after oral administration of ibuprofen tablets with slower absorption rates, as compared with rapidly absorbed formulations, is suggested to be indicative of presystemic inversion in the gastrointestinal tract (GIT) (8, 9). Direct evidence for inversion in the GIT has been provided for ibuprofen in humans (9) and benoxoprofen (10) and FN (7) in the rat.

The objective of this work was to investigate and further localize the site of chiral inversion in the GIT and demonstrate the involvement of the intestinal contents, the epithelium, and/or muscle layer of the gut wall. The rat was used as animal model (7)

and R-FN as a drug model (11).

2.2. Materials and Methods

2.2.1. Chemicals

Racemate and R enantiomer of FN calcium (FN) were obtained as gifts from Eli Lilly (Toronto, Canada). The stereochemical purity of the R-FN was 98% as measured using the stereospecific assay (16). Neomycin sulphate and erythromycin were purchased from the Sigma Chemical Company (St. Louis, MO, U.S.A.). Nutrient agar (NA) and Plate count agar (PCA) were obtained from Difco (Difco Laboratories, Detroit, Michigan, U.S.A.) and BBL (Becton, Dikinson and Company, Cockeysville, MD, U.S.A.), respectively.

2.2.2. Animals and Treatments

2.2.2.1. Rat Surgery and Tissue Preparations

Adult female Sprague-Dawley rats with body weight of 250-300 g were used for all parts of the study. The animal was anesthetized with diethyl ether and the upper jejunum was removed through a vertical midline incision. Jejunum as sheets, its contents, epithelial cells and muscle layer were used for in vitro incubation (n = 3).

2.2.2.2. Isolation of Epithelial Cells

The epithelium layer was isolated utilizing both the scraping (12) and shaking

methods (13) in a cold room (4°).

Scraping Method. Following removal, the upper jejunum was flushed with 20 mL of ice-cold isotonic NaCl and cut longitudinally. The formed sheet of the jejunum was spread out on an ice-cold glass surface so that the serosal side was in contact with the glass surface. The sheet was then washed with 10 mL of freshly prepared Tris Mannitol solution (300 mM D-mannitol and 10 mM Tris HCl in distilled water, pH 7). Using a microscope slide, the epithelium layer was gently scraped and immediately incubated with R-FN (550 mg n=3) (12).

Shaking Method. In the shaking method, the jejunum was cut into 7.5 cm segments and directly everted onto the plastic pegs which were securely placed in a specially designed container filled with 600 mL of continually oxygenated EDTA-mannitol buffer (pH 7.4, containing 2.5mM EDTA, 300 mM D-mannitol and 10 mM Tris-HCl). The container was vibrated at 1400 Hz for 15 min. The formed suspension was centrifuged in 20 mL test tubes at 1500 g for 3 min. The resultant pellets were collected and pooled together. Aliquots of 550 mg of epithelial cells and the remaining muscle layer (washed with 20 mL of oxygenated cold K-H buffer) were incubated separately. It has been shown that 75% of cells separated with this method are viable after 3 h. We did not use proteolytic enzyme to facilitate the detachment of epithelium from the sub-epithelium layer as they may reduce the metabolic activities of the cells (13).

2.2.2.3. Antibiotic Treated (ABT) and Control Rats

Four female Sprague-Dawley rats received 500 mg/kg b. i. d. of both erythromycin base and neomycin sulfate orally for 3 days (14) while control rats received 1 mL of distilled water. Animals had access to water and food ad libitum. Twelve hours before removal of the jejunum, rats were deprived of food but water was allowed. At the end of the starvation period the upper jejunum was excised and everted, as described above .One end of the jejunum was tied off with surgical thread. The formed sack was filled with drug free warm (37°) oxygenated K-H buffer and placed in a organ bath. This method was similar to the method used by Esposito et al. (15). Sterility tests, in two media, were performed for the jejunum contents obtained from ABT and control rats.

2.2.2.4. Culture Media Preparations

To ascertain gut sterility, 23 g of nutrient agar (NA) and or 23.5 g of plate count agar (PCA) medium were dissolved in 1 L of cold fresh distilled water and then sterilized by autoclaving at 121° for 15 min. After cooling, the media were dispensed into disposable petri dishes under aseptic conditions. Using a sterile swab, samples of the intestinal contents were taken from the ends of the excised jejunum, of both ABT and control rats, transferred onto the media and incubated at 37° for three days. Blank media (control) were also incubated under the above conditions.

2.2.2.5. In vitro Incubation and Sampling Jacketed warm (37°) glass organ baths were filled with 50 mL of continually oxygenated (O_2:CO_2 95:5) K-H buffer containing 5 mg/L of R-FN. Jejunum as sheets, intestinal contents, isolated epithelial cells, gut muscle layer, or everted rat jejunum sacks of ABT and control rats were placed in the organ bath and incubated for 3 h. For everted rat gut sacks, samples were taken from both serosal and mucosal sides. At the end of each experiment, samples were taken, centrifuged at 1500 g for 3 min, the supernatants placed in acid-washed tubes to prevent spontaneous hydrolysis of glucuronide FN (24, 25) and samples kept at -20° until analysis.

2.2.3. Assay and Sample Analysis

Concentrations of R- and S-FN were measured based on a previously reported stereospecific assay (16).

2.2.4. Data Analysis

The percent of inversion of R-FN was calculated as $\%R_{inv=}$ (S*/5) X 100 where S* is the total (intact + conjugated) concentration of S-FN. The unpaired two-tailed Student's t-test was used to determine the differences in inversion and conjugation between the intestinal contents and the gut wall, and between serosal and mucosal side concentrations of R and S-FN in jejunum of control and ABT treated rats. One-way ANOVA followed by Duncan's Multiple Range test were utilized to determine the differences in the extent of inversion and conjugation among epithelium, separated by the

scraping and shaking methods, and muscle layer. The level of significance was set at α =0.05. The results are expressed as mean $\pm$ standard deviations (SD).

2.3. Results

As depicted in Fig. 2-1, incubation of R-FN with jejunum sheets resulted in 30.7$\pm$5.1% inversion. The muscle layer exhibited 7.8$\pm$2.1% while the epithelial cells showed 37.5$\pm$4.7% inversion activities. Incubation of R-FN with intestinal contents, resulted in only 5.7$\pm$2.2% inversion. Treatment with antibiotics resulted in total eradication of intestinal microflora as reflected in the lack of microbial growth in the culture plates. The absence of microflora, however, did not affect the inversion efficiency of the everted jejunum sacks (Fig. 2-2). All specimens demonstrated significant glucuronidation activity; this ranged from 14.5$\pm$2.8% for muscle layer (Fig. 2-1) to 27.0$\pm$6.16% in the serosal side of the everted sacks of control rats (Fig. 2-2). Antibiotic treatment did not influence the conjugation activity of the gut wall to a significant degree. However, the intestinal contents showed 15.5$\pm$1.5% glucuronidation activity.

2.4. Discussion

Previous studies have shown that in both rats and humans R-FN undergoes extensive chiral inversion (7, 11). Rapid plasma clearance of R-FN and subsequent rise of active antipode levels after p.o. or i.v. administration, and almost complete urinary excretion of the administered racemic FN as the S isomer, suggest that chiral inversion

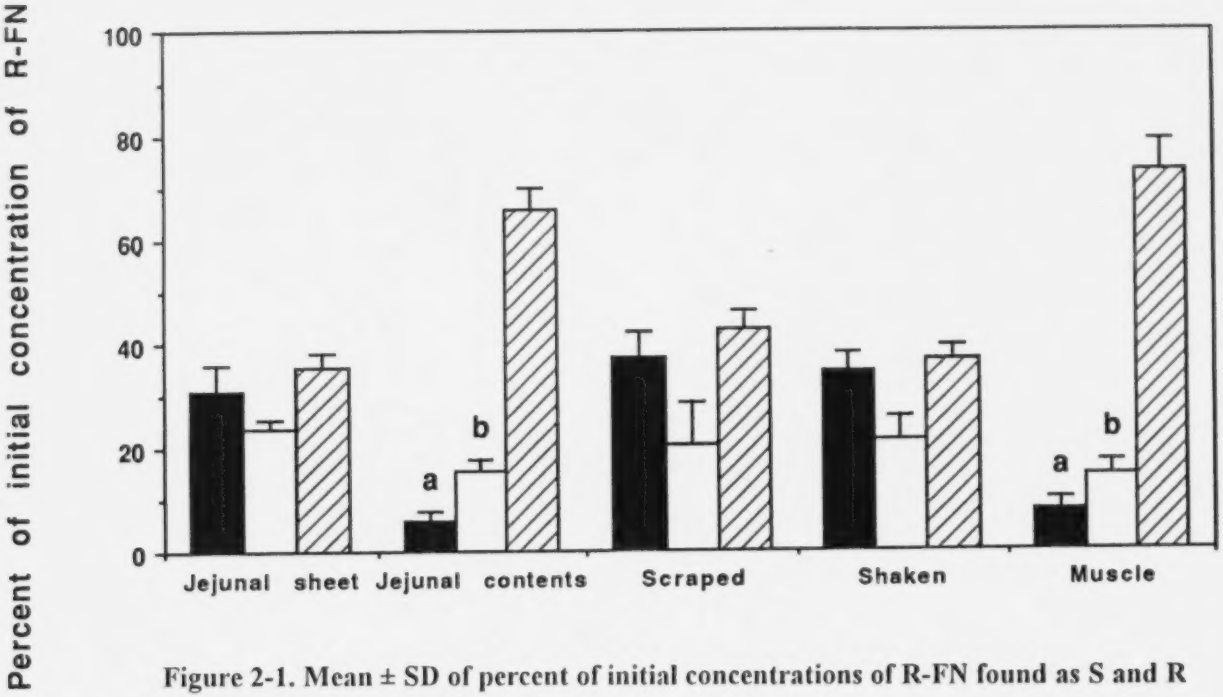


Figure 2-1. Mean ± SD of percent of initial concentrations of R-FN found as S and R isomers in jejunum sheet, intestinal contents, epithelial cells separated with scraping (Scraped) and shaking (Shaken) methods and muscle layer after 3 h incubation with 5 mg/L of R-FN. Keys; intact S, ■; conjugated R, □; and intact R, ⊠. a, significantly less inversion than other specimens. b, significantly less glucuronidation than other specimens.

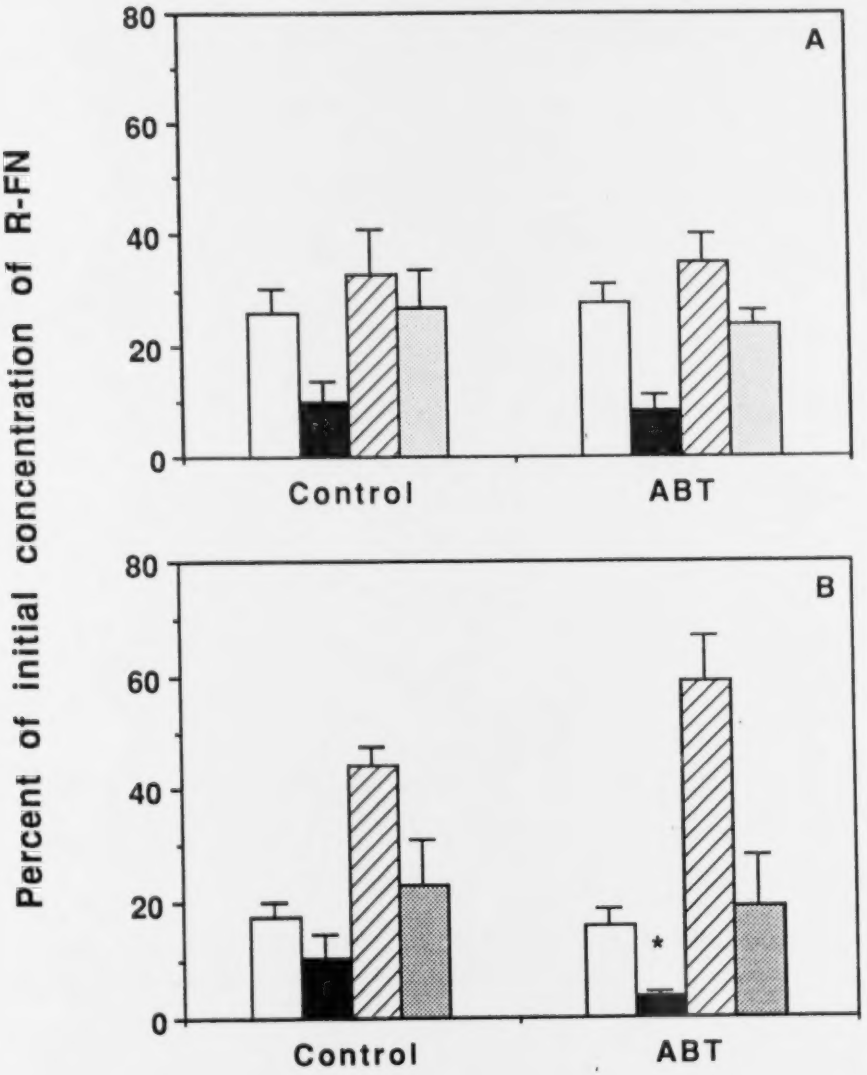


Figure 2-2. Percent of initial concentrations of R-FN found as intact and conjugated FN in the serosal (A), and mucosal (B) sides of jejunum sacks of control and antibiotic treated (ABT) rats after 3 h incubation with 5 mg/L of R-FN. Keys; Intact S, □; conjugated S, ■; Intact R, ⧄; conjugated R, ⊞.

***, significantly less glucuronidation than serosal side.**

is the main elimination pathway for R-FN. Presystemic as well as systemic inversion have been suggested for FN (7) and some other 2-APAs (17).

The GIT, as the first defensive barrier exposed to foreign compounds, is capable of performing phase I and phase II metabolic reactions (18). This, however, can be attributed to the metabolic activity of the gut wall, its contents and/or microflora (19). The mucosa of the intestine consists of three distinct layers: Epithelium, lamina propria and muscularis mucosa. Epithelium is made of a continuous one-cell-thick layer. The presence of numerous enzymes in the brush border barrier of the epithelial cells makes it the most metabolically active layer. Muscularis mucosa, which is a 3 to 10 cell-thick-layer of smooth muscle, separates the mucosa from submucosa. The metabolic activity of muscularis mucosa has not been studied thoroughly (20). Enzymes responsible for metabolism are mainly localized adjacent to the brush border barrier. However, it is not clear whether the enzymatic system responsible for the inversion process is located in the brush border barrier, mitochondria or endoplasmic reticulum. Recently, Tracy and Hall showed that mitochondria and cytosol of the rat liver hepatocytes were the most efficient subcellular locations for epimerizing R-IB-CoA thioester (21).

Our results indicate that epithelial cells were the most efficient site of intestinal bioinversion with negligible contribution from the muscle layer and intestinal contents. We used intact tissues or cells to investigate the inversion activity as other investigators (10, 22, 23) have shown it is more likely to detect the inversion activity when intact tissues or cells are used instead of sub-cellular fractions.

Similar extent of inversion before and after gut sterilization, coupled with the observed negligible inversion activity of the intestinal contents, suggest that the intestinal contents have no significant role in chiral inversion of FN.

Our results also indicate that the extent of inversion progressively increased during the incubation from 7% for the first h to a maximum of 40% after 3 h. This indicates the importance of residence time at the specific site of inversion. A similar pattern has been seen when R-IB was incubated in the presence of human gut (9).

Substantial amounts of glucuronidated R and S-FN were formed after incubation of R isomer in the presence of rat jejunum, intestinal contents, isolated epithelium and muscle layer. In most cases glucuronidation was stereoselective in favor of S isomer (Fig. 2-3). Stereoselectivity in acyl glucuronidation has been previously observed with other NSAIDs in human, monkey and rabbit (24) and rat (25).

It is important to note that in vitro conjugation of 2-APAs is dependent upon a variety of factors such as the substrate and its concentration, species used, method of tissue preparation, cell density, presence of oxygen, cofactors, composition of incubation medium and duration of incubation (26). It has also been reported that using anesthetic agents, such as ether, reduces the extent of glucuronidation activity in rat hepatocytes, however, this effect has been shown to be minimal for the intestinal epithelial cells (18).

More than 400 bacterial species, possessing a variety of enzyme activities, have been isolated from GIT or feces of healthy animals. Various microorganism exist in different intestinal segments and practically there is no sterile region in GIT. Generally,

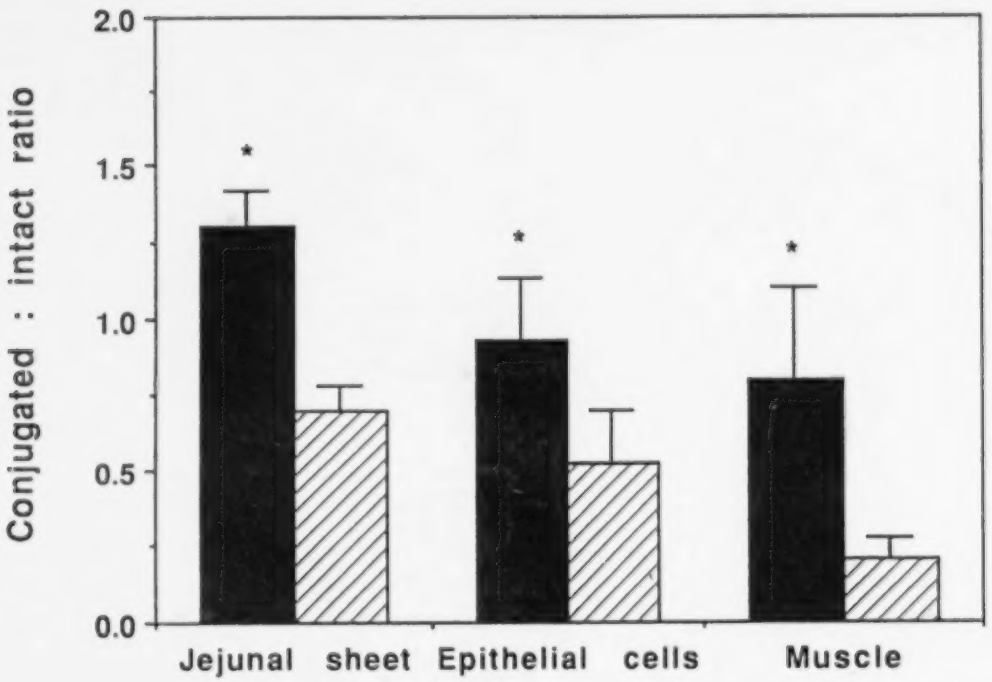


Figure 2-3. Ratios of the conjugated:intact S-FN (■) and R-FN (▨) in rat jejunum sheet, scraped epithelium cells and muscle layer after 3 h incubation with 5 mg/L of R-FN. *, significantly higher ratio than that of R-FN.

the number of microorganisms increases from the beginning of the small intestine towards the colon (19). Although many foreign compounds undergo reduction by gut microflora, phase II reactions, such as esterification, has also been reported (18, 19). While the metabolic activities of the gut wall are generally highest in the duodenum and jejunum and decreases posteriorly, bacterial biotransformation reactions are greatest in the colon. For many xenobiotics, however, the metabolism by the gut wall is quantitatively more important than that by microflora (18). Our data indicate that although inversion by the gut contents was negligible, FN glucuronidation activity was evident. However, Simmonds et al. could not detect any inversion for benoxaprofen in rat intestinal or cecal contents (10).

2.5. Conclusion

In conclusion, the major site for GIT FN inversion appeared to be the epithelial cells, with minimal activities for the muscle layer and intestinal contents. However, as with epithelial cells, the muscle layer and gut contents efficiently glucuronidate R- and S-FN.

2.6. References

1. A. J. Hutt, J. Caldwell: The metabolic chiral inversion of 2-arylpropionic acids- a novel route with pharmacological consequences. J. Pharm. Pharmacol. 35, 693-704 (1983).

2. J. Caldwell, A. J. Hutt and S. Fournel-Gigleux: The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. Biochem. Pharmacol. 37, 105-114 (1988).

3. R. D. Knihinicki, K. M. Williams and R. O. Day: Chiral inversion of 2-aryl propionic acid non-steroidal anti-inflammatory drugs: In vitro studies of ibuprofen and flurbiprofen. Biochem. Pharmacol. 38, 4389-4395 (1989).

4. R. Mehvar and F. Jamali: Pharmacokinetic analysis of the enantiomeric inversion of chiral non-steroidal antiinflammatory drugs. Pharm. Res. 5, 76-79 (1988).

5. S. R. Cox: Effect of route of administration on chiral inversion of R (-)-ibuprofen. Clin. Pharmacol. Ther. 43, 146 (1988).

6. F. Jamali and B. W. Berry: Pharmacokinetics of ibuprofen enantiomers in the rat: Effect of route of administration. Eur. J. Drug Metab. Pharmacokin. 15, (Suppl.) S402 (1990).

7. B. W. Berry and F. Jamali: Presystemic and systemic chiral inversion of R-(-)-fenoprofen in the rat. J. Pharmacol. Exper. Ther. 258, 695-701 (1991).

8. F. Jamali, N. N. Singh, F. M. Pasutto, A. S. Russell and R. T. Coutts:

Pharmacokinetics of ibuprofen in humans following oral administration of tablets with different absorption rates. Pharm. Res. 5, 40-43 (1988).

9. F. Jamali, R. Mehvar, A. S. Rusell, S. Sattari, W. W. Yakimets and J. Koo: Human pharmacokinetics of ibuprofen enantiomers following different doses and formulations: Intestinal chiral inversion. J. Pharm. Sci. 81, 221-225 (1992).

10. R. G. Simmonds, T. J. Woodage, S. M. Duff and J. N. Green: Stereospecific inversion of R-(-)- benoxaprofen in rat and man. Eur. J. Drug Metab. Pharmacokin. 5, 169-172 (1980).

11. A. Rubin, M. P. Knadler, P. P. K. Ho, L. D. Bechtol and R. L. Wolen: Stereoselective inversion of (R)-fenoprofen to (S)-fenoprofen in humans. J. Pharm. Sci. 74, 82-84 (1985).

12. P. K. Konturex, T. Brzozowski, S. T. Konturex and A. Dembinski: Role of epidermal growth factor, prostaglandins, and sulfhydryls in stress-induced gastric lesions. Gastroentrol. 99, 1607-1615 (1990).

13. J. R. Dawson and J. W. Bridges: Xenobiotic metabolism by isolated intestinal epithelial cells from guinea-pigs. Biochem. Pharmacol. 28, 3299-3305 (1979).

14. M. Mikov, J. Caldwell, C. T. Dolphin and R. L. Smith: The role of intestinal microflora in the formation of the methylthio adduct metabolites of paracetamol: Studies in neomycin pretreated and germ-free mice. Biochem. Pharmacol. 37, 1445-1449 (1988).

15. G. Esposito and T. Z. Casky: Extracellular space in the epithelium of rat's small

intestine. Am. J. Physiol. 226, 50-55 (1974).

16. R. Mehvar and F. Jamali: Stereospecific high-performance liquid chromatographic (HPLC) assay of fenoprofen enantiomers in plasma and urine. Pharm. Res. 5, 53-56 (1988).

17. F. Jamali: Pharmacokinetics of enantiomers of chiral non-steroidal anti-inflammatory drugs. Eur. J. Drug Metab. Pharmacokin. 13, 1-9 (1988).

18. K. Hartiala: Metabolism of hormones, drugs and other substances by the gut. Pharmacol. Rev. 53, 496-534 (1973).

19. K. F. Ilett, L. B. G. Tee, P. T. Reeves and R. F. Minchin: Metabolism of drugs and other xenobiotics in the gut lumen and wall. Pharmacol. Ther. 46, 67-93 (1990).

20. K. L. Audus, R. L. Bartel, I. J. Hidalgo and R. T. Borchardt: The use of cultured epithelial and endothelial cells for drug transport and metabolism studies. Pharm. Res. 7, 435-451 (1990).

21. T. S. Tracy and S. D. Hall: Metabolic inversion of (R)-ibuprofen: Epimerization and hydrolysis of ibuprofenyl-Coenzyme A. Drug Metab. Dispos. 20, 322-327 (1992).

22. J. M. Mayer, C. Bartolucci, J. M. Maitre and B. Testa: Metabolic chiral inversion of anti-inflammatory 2-arylpropionates: Lack of inversion in liver homogenates, and study of methine proton acidity. Xenobiotica. 18, 533-543 (1988).

23. S. M. Sanins, W. J. Adams, D. G. Kaiser, G. W. Halsted and T. A. Baillie: Studies on the metabolism and chiral inversion of ibuprofen in isolated rat hepatocytes. Drug Metab. Dispos. 18, 527-533 (1990).

24. M. Mouelhi, H. W. Ruelius, C. Fenselau and D. M. Dulik: Species-dependent enantioselective glucuronidation of three 2-arylpropionic acids. Naproxen, ibuprofen and benoxaprofen. Drug Metab. Dispos. 15, 767-772 (1987).

25. Y. Nakamura and T. Yamaguchi: Stereoselective metabolism of 2-phenylpropionic acid in rat I. in vitro studies on stereoselective isomerization and glucuronidation of 2-phenylpropionic acid. Drug Metab. Dispos. 15, 529-539 (1987).

26. S. Fournel-Gigleux, C. Hamar-Hansen, N. Montassim, B. Antonie, O. Mothe, D. Decolin, J, Caldwell and G. Siest: Substrate specificity and enantioselectivity of arylcarboxylic acids glucuronidation. Drug Metab. Dispos. 16, 627-634 (1988).

CHAPTER THREE

Absorption Rate Dependency of Ibuprofen Inversion in the Rat

3.1. Introduction

2-Arylpropionic acid derivatives (2-APA) are an important sub-group of non-steroidal antiinflammatory drugs (NSAID) being used worldwide to alleviate the symptoms associated with inflammatory diseases. Like other chiral 2-APAs, ibuprofen (IB) possesses a chiral centre in the propionic acid side chain and as a result has two enantiomers. Although the pharmacological effects are mainly attributed to the S enantiomer (1), IB is currently administered as the racemic compound (2).

Similar to some other members of the 2-APAs, IB undergoes a unique and unidirectional chiral inversion of R to S isomer in human and laboratory animals (3). Depending upon the drug and species used in the study, the extent of inversion varies widely (3, 4). In humans, rats and dogs, IB undergoes substantial bioinversion (3, 5, 6, 7). The gastrointestinal tract (GIT), liver and kidney have been suggested as sites of chiral inversion for 2-APAs (3). For IB, in addition to the systemic inversion (25, 23), the GIT is suggested to be the presystemic site of inversion (4, 8). Cox et al. (9) have reported negligible inversion of R-IB in isolated rat liver. This observation further supports the notion that GIT may be the presystemic site of inversion for IB. Although previous studies (5, 8, 10) suggested that the extent of inversion with orally administered IB in human was correlated with the Tmax of the dosage form, the possibility of presystemic intestinal chiral inversion has been controversial.

Hence, the aim of this study was to investigate the in vivo and in vitro chiral inversion of IB in more detail and further delineate the site of presystemic inversion.

3.2. Materials and Methods

3.2.1. Chemicals

Racemic IB and fenoprofen calcium (FN, as internal standard, IS), were kindly supplied by Upjohn Co. of Canada (Don Mills, Ont., Canada) and Eli Lilly (Toronto, Canada), respectively. Enantiomers of IB, 99% stereochemically pure, as quantified using the stereospecific assay (16), were generously donated by KaliChemie Pharma GmbH (Hannover, Germany). (R)-(+)-1-(1-phenyl)ethylamine (PEA) was purchased from Sigma Chemical Company (St. Louis, MO.,USA). Ethyl chloroformate (ECF), isooctane, acetonitrile (ACN) (Assurance grade), hexane and isopropanol (Assurance grade) were obtained from BDH Chemicals Canada (Edmonton, Canada). Analytical grade triethylamine (TEA) and chloroform were obtained from Fisher Scientific (Fair Lawn, NJ. USA).

3.2.2. In vivo Study

3.2.2.1. Preparation of Dosage Forms

Oral solutions and suspensions of racemic IB were made, immediately before dosing the animals, by dissolving the powder in distilled water (adjusted to pH 8 with sodium bicarbonate) and suspending the powder in a 2% methyl cellulose, respectively. Sustained release granules were gifts from Apotex (Weston, Ont., Canada). Granules of 40-60 mesh size were used for in vivo study.

3.2.2.2. Animals, Dosing and Sampling

Male Sprague-Dawley rats weighing 250-300 g were used. In order to cannulate the jugular vein, the animals were anesthetized initially with diethyl ether and then maintained on methoxyflurane (Metofen, Pitman-Moore Ltd., Mississauga, Ont., Canada). The polyethylene tubing tipped with silastic tubing, was utilized for cannulation. After an overnight recovery, animals were fasted for 12 h while water was allowed ad libitum. Rats (n=4 in each group) were orally administered IB solution, suspension or sustained release granules (equivalent to 20 mg/kg of racemate). A flexible plastic tubing with internal diameter of 2.5 mm was used to introduce the sustained release granules into the stomach. Food was allowed 3 h after the dosing. Blood samples (0.2 mL) were drawn via the jugular vein at 0, 0.17, 0.33, 0.5, 1, 2, 3, 4, 6 and 8 h post dose, centrifuged for 3 min, and the plasma separated and kept at -20° until analyzed. After each blood sampling, the cannula was flushed with 0.1 mL of heparin (100 unit/mL).

3.2.3. In vitro Study

3.2.3.1. Animals and Tissue Preparations

Male Sprague-Dawley rats (n=4) were anesthetized with diethyl ether and 15 cm of the jejunum, ileum and colon were removed through a vertical midline incision. The animals were then sacrificed by cardiac puncture. Gut segments were flushed with 20 mL of cold isotonic NaCl to remove the intestinal contents, placed in ice-cold

Krebs-Henseleit (K-H) buffer, pH 7.4, for 5 min and then everted using a glass rod (11).

3.2.3.2. In vitro Incubation of R-IB with Rat Intestine

The rat gut segments were incubated in a jacketed glass organ bath (37°), which was filled with continually oxygenated (O_2:95, CO_2:5) K-H buffer containing 5 mg/L of R-IB for 3 h. Samples were collected at the end of the experiment and centrifuged at 1500 g for 3 min. In order to prevent spontaneous hydrolysis of labile IB acyl glucuronide, the supernatant was stored in acid-washed test tubes at -20° until analyzed (12, 13). To evaluate the viability of the gut during in vitro incubation, one segment of the gut was simultaneously incubated with R-FN as a positive control for inversion as it undergoes substantial bioinversion in the rat intestine (14).

3.2.3.3. In vitro Incubation of R-IB with Human Gut

In order to directly examine the possibility of inversion by human gut, we incubated R-IB with gut tissues obtained from cancer patients (47-, 76-, and 81-year-old male subjects) who required partial removal of ileum or colon. The two ends of the excised segments (one ileum and two colons) that were not afflicted were used. The tissues were immediately placed in ice-cold K-H buffer solution, weighed (5.3-9.3 g) and then incubated with R-IB in organ bath. The time period between removal and start of incubation did not exceed 35 min. Formation of the S enantiomer was evaluated in samples collected at 5, 15, 30, and 45 min and 1, 1.5, 2, and 3 h after commencement

of incubation. In addition, to examine the effect of homogenization, a 7.2 g segment of ileum was immediately homogenized in two volumes of K-H solution and incubated as described above.

3.2.4. Stereospecific Assay for IB Enantiomers

There are a number of gas chromatographic and high-performance liquid chromatographic (HPLC) methods available for the separation and quantitation of IB enantiomers from biological fluids (15). Many of these methods, however, require lengthy precolumn derivatization and sample preparation, or lack sufficient sensitivity. We initially used a reversed-phase method, previously established in our laboratory, employing S-(-)-1-(1-naphtyl)ethylamine as precolumn derivatizing agent. The method resulted in the co-extraction of numerous endogenous compounds, some of which interfered with the quantitation of IB enantiomers when inefficient columns were used (15). Hence, attempts were made to improve the assay (16).

3.2.4.1. Standard Solutions

Solutions of 50 mM TEA and 6 mM ECF were prepared in ACN, while PEA (0.5 M) was dissolved in ACN/TEA (8:2 v/v). Standard solutions of racemic IB (100 μg/mL) and racemic FN (25 μg/mL) were prepared by dissolving the powders in 0.01

M NaOH in HPLC grade water. These solutions were kept at 4° in order to increase their stability.

3.2.4.2. Sample Preparations

To an aliquot of biological sample, were added 50 μl of IS (25 μg/mL of ($\pm$) FN solution) and 0.2 mL of 0.6 M H_2SO_4. The drug and IS were subsequently extracted into 3 mL of isooctane:isopropanol (95:5) followed by vortexing for 30 sec and centrifuging at 1800 g for 5 min. The organic layer was transferred to clean, dry test tubes and evaporated to dryness under vacuum. To the remaining residue, were added 200 μl of 50 mM TEA (to dissolve the drug and IS), 50 μl of ECF (to form the mixed anhydride) and after 1 min, 50 μl of 0.5 M PEA (to form the corresponding PEA-IB and PEA-FN diastereoisomers). Four minutes later, the derivatization reaction was stopped by the addition of 1 mL HCl (0.25 N) and the diastereoisomers extracted into chloroform. The chloroform layer was evaporated and the residue was reconstituted in 200 μL of mobile phase and aliquots of 10-150 μl were injected to HPLC system.

3.2.4.3. Apparatus and Chromatographic Conditions

The HPLC apparatus (Waters, Mississauga, Canada) consisted of a Model 590 pump, a Model 481 variable-wavelength ultraviolet detector (set at 225 nm), a 710B WISP autosampler, a 2 cm x 4.6 mm I.D. Uptight precolumn (Upchurch Scientific, Rexdale, Canada) packed with 37-53 μm reversed-phase packing material and a 4.6 mm

x 10 cm analytical column containing 5 μm particles of ODS (C18) packing material (partisil 5 ODS, Phenomenex, Torrance, CA, USA). The mobile phase consisted of ACN-water-acetic acid-TEA (46.5: 53.5: 0.1: 0.03), pH 4.9, and was pumped at a flow rate of 1.6 mL/min at ambient temperature. The corresponding diastereoisomeric peaks of IS and IB were monitored by a UV detector at 225 nm.

3.2.5. Data Analysis

The elimination rate constants of the terminal phase (ß) of the IB isomers were estimated using linear regression of the log-linear terminal phase of the plasma concentration vs time curve (4, 6 and 8 h data points). The areas under the concentration-time curves (AUC) were estimated utilizing the linear trapezoidal method. $AUC_{(0-\infty)}$ were calculated as the summation of $AUC_{(0-8)}$ and $AUC_{(8-\infty)}$. The differences between mean observations were determined using one-way ANOVA followed by the Duncan's Multiple Range test. The unpaired two-tailed Student's t-test was used to evaluate the stereoselective glucuronidation of IB isomers in each segment. Pearson linear correlation was used to determine the relationship between the S:R AUCs and the corresponding Tmaxs of the R enantiomer. The percent of in vitro inversion ($\%R_{inv}$) of R-IB was calculated as $\%R_{inv}=(S^*/5) \times 100$ where S* is the total (intact + conjugated) concentration of S-IB. The data are expressed as mean $\pm$ standard deviation (SD) unless otherwise stated. The statistical significance was set at $\alpha = 0.05$.

3.3. Results

3.3.1. In vivo Study

As depicted in Fig. 3-1 and Tab. 3-1, in all cases the AUCs of S-IB were significantly higher than those of R-IB. After administration of sustained release granules, the AUC of R-IB and the Cmax of S- and R-IB were significantly lower. However, the Tmaxs were significantly longer and S:R AUC and S:R concentration ratios were significantly higher than those of suspension and/or solution. Neither $t_{1/2}$ nor ß of R- and S-IB were significantly different after administration of 3 dosage forms. When S:R AUC and the corresponding Tmax of R-IB were plotted (Fig. 3-2), a significant and positive correlation was found ($r=0.82$). As compared with solution and suspension, the total (S+R) AUC of sustained release granules was lower, however, the differences did not reach significant level.

3.3.2. In vitro Study

As illustrated in Fig. 3-3, after 3 h incubation, rat ileum and jejunum showed significantly higher R to S chiral bioinversion activity than colon. However, the differences between ileum and jejunum were not significant. Conversely, the extent of glucuronidation for R-IB was significantly higher in colon compared to jejunum and ileum. In the case of S-IB, on the other hand, all segments gave the same degree of glucuronidation. Stereoselective glucuronidation in favour of S-IB was found in jejunum and ileum but not in the colon (Fig. 3-4). Jejunum, ileum and colon were capable of

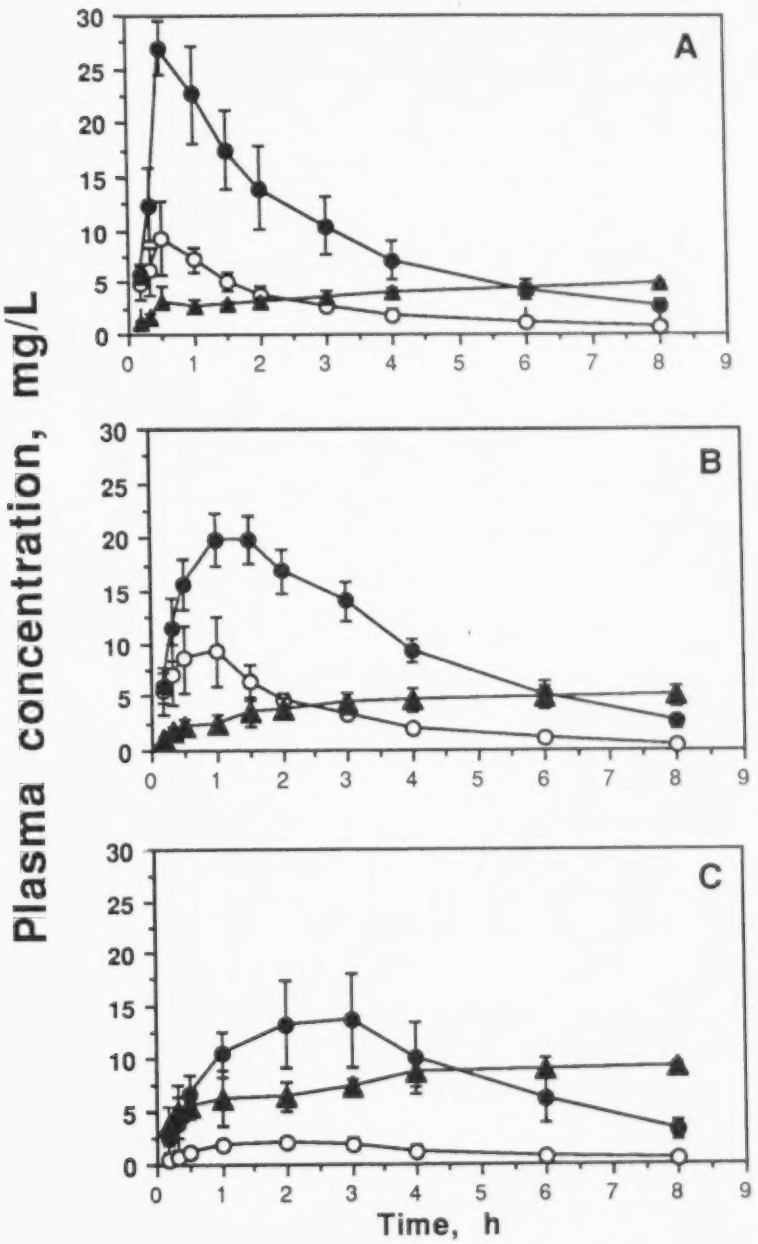


Figure 3-1: Plasma concentration-time courses of IB enantiomers after oral administration of 20 mg/kg of racemic compound as a solution (A), suspension (B), and sustained release granules (C). Keys: R-IB, ○; S-IB, ●; and S:R concentration ratio, ▲.

Table 3-1: Mean (SD) of pharmacokinetic indices of IB after oral administration of 20 mg/kg of racemate as solution, suspension, or sustained release granules in the rats.

	R-IB				S-IB			
	Cmax	Tmax	$t_{1/2}$	AUC	Cmax	Tmax	$t_{1/2}$	AUC
Dosage form	mg/L	h	h	mg.h/L	mg/L	h	h	mg.h/L
Solution	10.2	0.6	2.7	24.6	28.0	0.5	2.7	85.3
	(2.4)	(0.2)	(0.4)	(3.8)	(2.8)	(0.2)	(0.5)	(15.3)
Suspension	9.3	1.0	2.3	27.6	21.1	1.4	2.2	94.9
	(3.8)	(0.0)	(0.3)	(6.7)	(2.3)	(0.2)	(0.2)	(7.2)
SR granules	2.2*	2.3*	2.3	10.5*	14.1*	2.5*	2.5	77.7
	(0.4)	(0.2)	(0.3)	(3.2)	(5.4)	(0.6)	(0.4)	(30.4)

*, significantly different from those of solution and suspension.

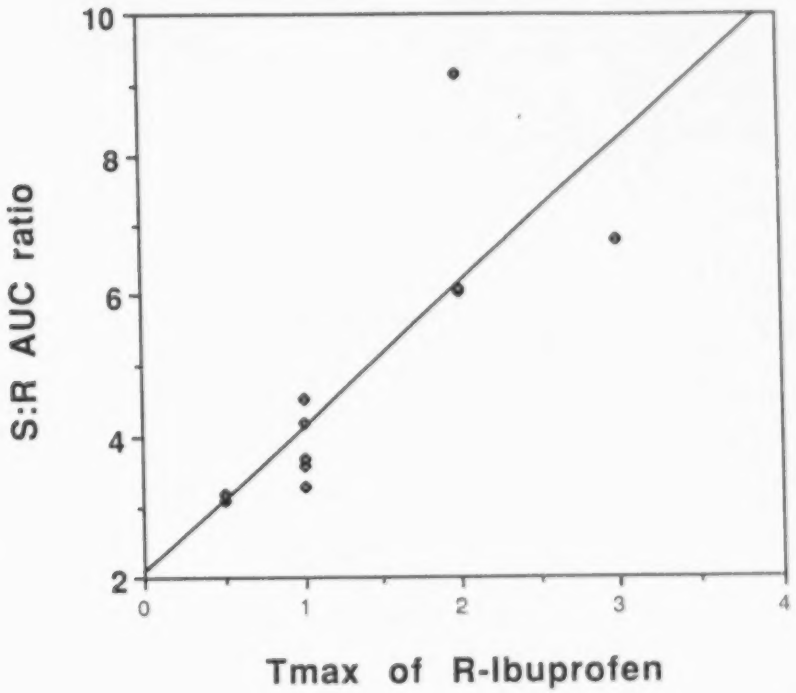


Figure 3-2: Relationship between S/R AUC ratios and the corresponding Tmaxs of R-IB after administration of three different dosage forms. The two variables are significantly and positively correlated (r=0.82).

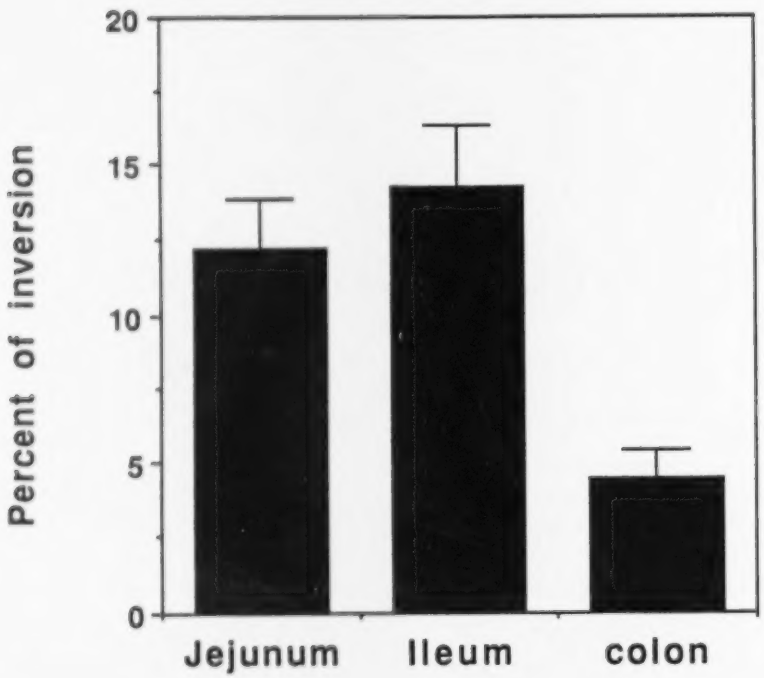


Figure 3-3: The extent of in vitro inversion of R-IB in the presence of different segments of rat intestine after 3 h incubation at 37^{o} (n=4).

***;significantly less inversion than jejunum and ileum.**

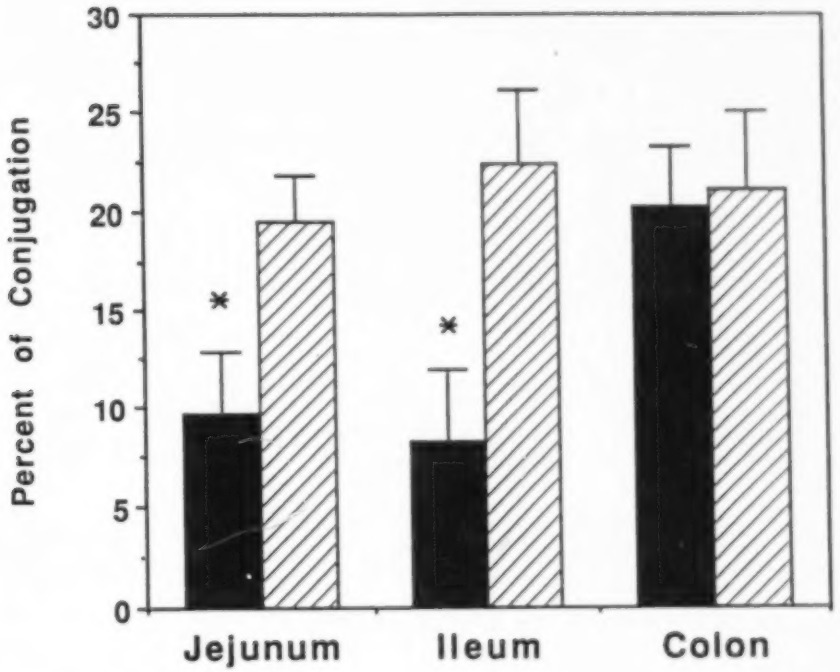


Figure 3-4: The extent of in vitro glucuronidation of IB enantiomers by different segments of rat intestine after 3 h incubation at 37°

Keys: R-IB, ■ and S-IB, ⊠.

***, significantly different from R-IB.**

inverting R-FN (positive control) to its S configuration by 18, 23 and 10%, respectively. The time course of inversion and glucuronidation of IB during incubation with R-IB in the presence of human colon is shown in Fig. 3-5. Chiral inversion was evident 15 min after commencement of incubation with both colon and ileum. After 3 h, the extent of inversion was 33.0% for ileum and 20.0% and 34.5% for two colons, respectively. No inversion was evident using fresh homogenates of ileum under the above conditions. The enantiomers were found as intact and conjugated forms. The extent of glucuronidation was negligible after incubation with ileum but was 20.0% and 23.3% for R-IB and 11.5% and 13% for S-IB in the presence of colons. The concentration of conjugated R-IB reached its maximum in 40 to 60 min, after which a gradual decline was observed (Fig. 3-5).

3.4. Discussion

Stereoselective disposition of ibuprofen has been attributed mainly to a unidirectional metabolic chiral inversion of R-IB to its antipode (3). The involvement of both systemic (25) and presystemic processes (5) in bioinversion of IB has been suggested. However, the possibility of intestinal presystemic inversion and its absorption rate dependency has been a matter of controversy among investigators (4, 17, 29).

Subsequent to initial reports suggesting an absorption rate dependent chiral inversion for IB (5, 8, 10), many attempts have been made to examine the involvement of presystemic process in IB chiral inversion. In many of these studies, however, the

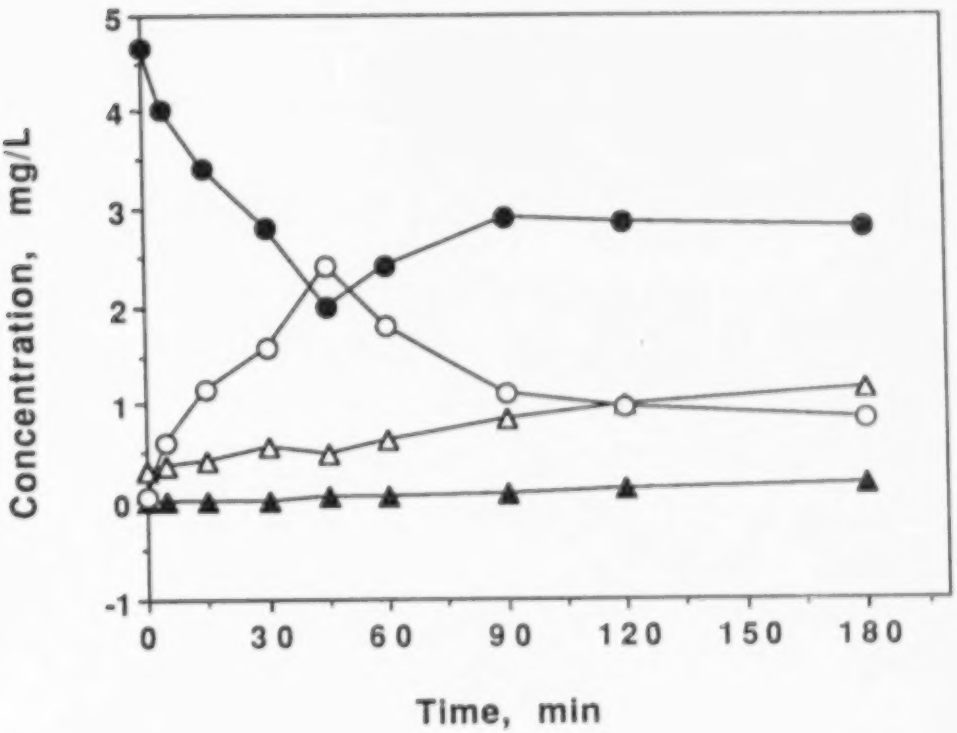


Figure 3-5: Inversion and conjugation of IB during incubation of R-IB in presence of an excised segments of human colon. Keys: S-IB, Δ; conjugated S-IB, ▲; R-IB, ●; conjugated R-IB, ○.

extent of presystemic inversion has been investigated by comparing i. v. administration with rapidly absorbed oral formulations (23, 25, 28, 29). Such an approach ignores the fact that, as has been shown earlier (23, 25), a product with a rapid absorption rate is expected to give an S:R AUC ratio similar to that of an i. v. dose. A significantly higher than unity S:R AUC ratio is evident only when Tmax of the product is prolonged (5, 8, 10). Thus, comparing an i. v. dose with a rapidly absorbed oral formulation cannot resolve the controversy. The lack of difference in S:R AUC ratios and/or fraction inverted reported between i. v. and rapidly absorbed oral formulations (23, 25, 28, 29) can be attributed to the short residence time of the oral product in the intestine, the proposed site of presystemic inversion. Our findings show that a reduction in the absorption rate of IB results in relatively higher concentrations of S-IB (Fig. 3-1) as indicated by higher S:R AUC ratios (Fig. 3-2). Similar results have been reported earlier after administration of IB formulations with different rates of absorption (5, 8, 10).

The positive correlation between the S:R AUC ratio and Tmax of R-IB, observed in the rat (Fig. 3-2), has also been reported in humans (8). These observations are clear indicators of the dependency of presystemic chiral inversion upon the rate of absorption. Moreover, the rat appears to serve as a suitable animal model for investigation of presystemic inversion of IB.

The higher S:R AUC ratios seen after delay in the absorption of IB could be due to stereoselective absorption of enantiomers and/or an increase in presystemic chiral inversion in liver and/or intestine. The former is an unlikely explanation since absorption

of IB seems to be a passive process; hence, absorption is improbable to be stereoselective (27). It is also unlikely for the liver to be the major site of presystemic inversion, as Cox et al. (9) detected very limited chiral inversion after perfusion of R-IB into the in situ recirculating perfused rat liver. Similarly Mayer et al. (24) were not able to detect inversion in liver homogenates. Low extraction ratio (0.04)(9), coupled with relatively high clearance of R-IB (5), indicates the involvement of other organs, such as intestine, in the presystemic inversion process. In addition, it has been reported that the extent of inversion in humans remains constant after oral administration of 50 to 1200 mg IB (5) or tablets ranging from 200 to 1200 mg (18). This explains that the extent of inversion is dependent upon the absorption rate rather than the size of the dose. Therefore, the intestine rather than the liver seems to be the organ responsible for presystemic bioinversion of IB.

Recently, Jeffrey et al. (19) attempted to examine the possibility of a presystemic inversion for IB using rat in situ intestine/liver perfusion. They suggested the liver, but not intestine, as the site of inversion. The lack of inversion (19) , however, may be due to the fact that Jeffrey et al. used a solution of IB which is absorbed very rapidly. Hence, insufficient time is allowed for inversion. Moreover, they did not mention whether the perfusate was oxygenated and if they considered the possibility of glucuronidation of formed S-IB. In our study, after 3 h of in vitro incubation of R-IB in the presence of human and rat intestine, considerable inversion, as well as glucuronidation, were evident (Fig. 3-3 and 3-5). These observations further support the notion of presystemic

inversion in the intestine. We have previously reported that in vitro gut inversion for both IB in humans (5) and FN in rats (26) was a time-dependent process and the intestinal epithelium layer was the main site of inversion for FN in the rat gut (26).

Moreover, our data showed stereoselectivity in glucuronidation of IB in favour of S-isomer in rat jejunum and ileum but not in colon. On the other hand, glucuronidation of IB favored R-IB in human colon. This may be indicative of the presence of a stereoselective process involving glucuronidation in different segments of the gut (20, 21). Enantioselective glucuronidation in favor of S-IB has also been reported in humans by Lee et al.(22).

It is interesting to note that when total $AUC_{(S+R)}$ or AUC_{R} was considered, the reduction in the absorption rate resulted in a lower relative bioavailability for sustained release formulation ($F_{(S+R)}=0.72$ and $F_{R}=0.44$) as compared to the oral solution. The apparent bioavailability of the S enantiomer, however, remained unaffected as a significant difference was not found between the S AUCs of the three dosage forms (Tab. 3-1). This indicates a greater extent of chiral inversion for the sustained release formulation as compared to the rapidly absorbed oral solution.

The extent of presystemic inversion may vary significantly when a physical mixture of pure enantiomers and/or racemic compound of IB are given as solid dosage forms because the dissolution rate of enantiomers is significantly different from the racemic compound (2). Hence, the assumption giving the mixture of pure isomers is exactly as giving racemate used in some studies may not hold valid. Nevertheless, this

assumption is valid if the physical mixture or compound is given in the form of a solution form.

3.5. Conclusion

The extent of IB inversion is positively and significantly correlated with the Tmax of the dosage forms due to the presystemic chiral inversion of delayed absorption IB formulations. Different segments of human and rat intestine are capable of inverting and glucuronidating IB. The rat may serve as a suitable animal model for studying pharmacokinetics of IB.

Our data do not rule out the possibility of systemic inversion of IB.

3.6. References

1. A. J. Hutt and J. Caldwell: The importance of stereochemistry in the clinical pharmacokinetics of the 2-arylpropionic acid non-steroidal anti-inflammatory drugs. Clin. Pharmacokin. 9, 371-373 (1984).

2. S. K. Dwivedi, S. Sattari, F. Jamali and A. G. Mitchell: Ibuprofen racemate and enantiomers: Phase diagram, solubility and thermodynamic studies. Int. J. Pharm. Sci. 87, 95-104 (1992).

3. F. Jamali: Pharmacokinetcs of enantiomers of chiral non-steroidal anti-inflammatory drugs. Eur. J. Drug Metab. Pharmacokin. 13, 1-9 (1988).

4. R. Mehvar and F. Jamali: Pharmacokinetic analysis of the enantiomeric inversion of chiral nonsteroidal antiinflammatory drugs. Pharm. Res. 5, 76-79 (1988).

5. F. Jamali, R. Mehvar, A. S. Russell, S. Sattari, W. W. Yakimets and J. Koo: Human pharmacokinetics of ibuprofen enantiomers following different doses and formulations: Intestinal chiral inversion. J. Pharm. Sci. 81, 221-225 (1992).

6. D. G. Kaiser, G. J. Vangissen, R. J. Reischer and W. J. Wechter: Isomeric inversion of ibuprofen (R)-enantiomer in humans. J. Pharm. Sci. 65, 269-273 (1976).

7. H. Y. Ahn, G. L. Amidon and D. E. Smith: Stereoselective disposition of ibuprofen in the dog. Pharm. Res. 8, 1186-1190 (1991).

8. F. Jamali, N. N. Singh, F. M. Pasutto, A. S. Russell and R. T. Coutts: Pharmacokinetics of ibuprofen enantiomers in humans following oral

administration of tablets with different absorption rates. Pharm. Res. 5, 40-43 (1988).

9. J. W. Cox, S. R. Cox, G. Vangiessen and M. J. Ruwart: Ibuprofen stereoisomer hepatic clearance and distribution in normal and fatty in situ perfused rat liver. J. Pharmacol. Exp. Ther. 232, 636-643 (1985).

10. M. T. Borin, S. R. Cox, J. M. Hageman and G.J. Szpunar: Serum concentrations of ibuprofen enantiomers following multiple dose administration of sustained release ibuprofen 800 mg tablets or Motrin 400 mg tablets. J. Pharm. Res. 10, (Suppl.) 159S (1988).

11. G. Esposito and T. Z. Csaky: Extracellular space in the epithelium of rat's small intestine. Am. J. Physiol. 226, 50-55 (1974).

12. P. C. Smith, J. Hasegava, N. J. Landgenijk and L. Z. Benet: Stability of acyl glucuronides in blood, plasma and urine: Studies with zomepirac. Drug Metab. Dispos. 13, 110-112 (1985).

13. R. A. Upton, J. N. Buskin, R. L. Williams N. H. G. Holford and S. Riegelman: Negligible excretion of unchanged ketoprofen, naproxen and probenecid in urine.J. Pharm. Sci. 69, 1254-1257 (1980).

14. B. W. Berry and F. Jamali: Presystemic and systemic chiral inversion of R-(-)-fenoprofen in the rat. J. Pharmacol. Exp. Ther. 258, 695-701 (1991).

15. R. Mehvar, F. Jamali and F. M. Pasutto: Liquid chromatographic assay of ibuprofen enantiomers in plasma. Clin. Chem. 34, 493-496 (1988).

16. M. R. Wright, S. Sattari, D. R. Brocks and F. Jamali: Improved high-performance liquid chromatographic assay for enantiomers of ibuprofen. J. Chromatogr. 583, 259-265 (1992).

17. J . Romero, R. J. Rackley and C. T. Rhodes: An evaluation of ibuprofen bioinversion by simulation. Chirality 3, 418-421 (1991).

18. A. M. Evans, R. L. Nation, L. N. Sansome, F. Bochner and A. A. Somogyi: The relationship between the pharmacokinetics of ibuprofen enantiomers and the dose of racemic ibuprofen in humans. Biopharm. Drug Dispos. 11, 507-518 (1990).

19. P. Jeffrey, G. T. Tucker, A. Bye, H. K. Crewe and P. A. Wright: The site of inversion of R(-)-ibuprofen: Studies using rat in-situ isolated perfused intestine/liver preparations. J. Pharm. Pharmacol. 43, 715-720 (1991).

20. A. Sj. Koster and J. Nordhoek: Glucuronidation in the rat intestinal wall. Biochem. Pharmacol. 32, 895-900 (1983).

21. O. Hanninen, A. Aitio and K. Hartiala: Gastrointestinal distribution of glucuronide synthesis and relevant enzymes in the rat. Scan. J. Gastroenterol. 3, 461-464 (1968).

22. E. J. D. Lee, K. Williams, R. Day, G. Graham and D. Champion: Stereoselective disposition of ibuprofen enantiomers in man. Br. J. Clin. Pharmacol. 19, 669-674 (1985).

23. F. Jamali and B. W. Berry: Pharmacokinetics of ibuprofen enantiomers in the rat: effect of route of administration. Eur. J. Drug Metab. Pharmacokin. 15, (Suppl.) S-402 (1990).

24. J. M. Mayer, C. Bartolucci, J. M. Maitre and B. Testa: Metabolic chiral inversion of anti-inflammatory 2-arylpropionates: Lack of reaction in liver homogenates and study of methine proton acidity. Xenobiotica, 18, 533-543 (1988).

25. S. R. Cox: Effect of route of administration on chiral inversion of R(-)-ibuprofen. Clin. Pharmcol. Ther. 43, 146 (1988).

26. S. Sattari and F. Jamali: Chiral inversion and glucuronidation of R-(-)-fenoprofen in the rat gut. (Submitted for publication to Chirality 1993)

27. F.Jamali, R. Mehvar and F. M. Pasutto: Enantioselective aspects of drug action and disposition: Therapeutic pitfalls. J. Pharm. Sci. 78, 695-715 (1989).

28. W. S. Beck, G. Geisslinger, H. Engler and K. Brune: Pharmacokinetics of Ibuprofen Enantiomers in Dogs. Chirality 3, 165-169 (1991).

29. S. D. Hall, A. C. Rudy, P. M. Knight and D. C. Brater: Lack of presystemic inversion of (R)- to (S)-ibuprofen in humans. Clin. Pharmacol. Ther. 53, 393-400 (1993).

CHAPTER FOUR

General Discussion and Conclusions

4. General Discussion and Conclusions

Despite the structural similarities, antipodal isomers are often subjected to different metabolic pathways mainly due to stereoselective or stereospecific enzyme actions. The chiral bioinversion of the R isomer of some 2-APAs is an intriguing example. Presystemic as well as systemic inversion have been suggested to be involved in this process. Presystemic inversion may take place in the GIT and/or liver. The exact site of inversion in the intestine and also the rate dependency of presystemic bioinversion are examined in this thesis.

Absorption rate dependency of the extent of IB has been reported in humans, however, there is a controversy about the organ(s) responsible for presystemic inversion. Therefore, IB was used to investigate the correlation between the absorption rate and the extent of presystemic inversion and, further, to localize the organ responsible for the presystemic inversion. In order to better localize the site of inversion in the rat gut, FN was used as its inversion is substantial in rat jejunum. The rat was used as animal model.

It has been previously reported that the extent of FN inversion was the highest in rat jejunum. Thus, in order to better localize the site of intestinal inversion, rat jejunum, its contents, isolated epithelial cells and muscle layer were incubated with R-FN. In addition, utilizing the jejunum of antibiotic treated and control rats, the extent of inversion and glucuronidation was examined in the absence of microbial flora.

The ability of the GIT to metabolize xenobiotics can be attributed to the gut wall, its contents and/or microflora. In the gut wall, the epithelial cells have been considered to be the most efficient layer in gut metabolism because of the presence of a variety of enzymes in the brush border barrier, mitochondria and endoplasmic reticulum. GIT microflora are also capable of metabolism with greatest activity in the colon. However, for many drugs the extent of metabolism by the gut wall is much higher than that of microflora.

Our data revealed that indeed the epithelium layer was the main site of chiral inversion while contribution of the muscle layer and gut contents were negligible in the process. We used intact tissues or cells instead of subcellular fractions as they are more likely to detect inversion activity. In addition, similar to the epithelial cells, glucuronidation by muscle and intestinal contents was evident.

In chapter three, we examined the absorption rate dependency of inversion of IB by giving three different oral dosage forms. Moreover, R-IB was incubated with different segments of the rat gut and human colon and ileum to further investigate the possibility of intestinal presystemic inversion.

The involvement of a presystemic process in the chiral inversion has been examined, however, the results are not consistent due to the fact that some investigators compared i. v. injection with rapidly absorbed oral dosage forms and used the data as a discriminative measure and could not find any significant difference in S:R AUC ratios. On the other hand, when products with different rates of absorption were compared, the

difference became significant. These observations suggest that comparing the S:R AUCs after i. v. and rapidly absorbed formulation is not a proper way to resolve the controversy.

We realized that a reduction in the absorption rate resulted in significantly higher S:R AUC ratios. These variables were also significantly and conversely correlated. These observations are clearly suggestive of the existence of absorption rate dependency in the inversion process.

Low hepatic extraction ratios and almost negligible inversion by the liver coupled with the lack of difference in S:R AUC ratios after i.v. and rapidly absorbed oral dosage form administration provide important evidence that the intestine is the site of presystemic inversion for IB. When R-IB was incubated with rat and excised human intestinal segments, their ability in performing inversion and glucuronidation was evident. This again supports the possibility of the intestinal presystemic inversion.

In summary;

1. The epithelial cells were the major site of R-FN inversion with minimal contribution of intestinal contents, muscle layer and microflora of the rat intestine.

2. As with epithelial cells, the muscle layer and intestinal contents were active in glucuronidation of FN.

3. The extent of IB inversion was significantly correlated to the Tmax of the dosage form.

4. The rat intestine is the major site of presystemic inversion for slowly absorbed

IB formulations. Human and rat gut were also capable of performing inversion and glucuronidation of IB.

5. Relative bioavailability of IB was lower when the absorption was delayed, however, the AUC of the active S isomer was not changed to a significant degree mainly due to a greater extent of R to S inversion.

6. Utilizing a stereospecific assay is essential when assessing bioequivalency and clinical efficacy of IB dosage forms whose absorption rates differ.